Archives of Internal Medicine

VOLUME 41 MARCH, 1928 NUMBER 3

THE SPECIFIC TREATMENT OF LOBAR PNEUMONIA *

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Lobar pneumonia now ranks with tuberculosis in respect to mortality rate. In the registration area of the United States, the annual toll of deaths for each of these infectious diseases is approximately 100,000. While in both instances this figure is considerably lower than it was twenty years ago, it is still high enough to make them by far the most deadly of the infectious diseases. It is interesting that both of these diseases should be largely pulmonary. This fact suggests that there may be something in the anatomic structure of the lung which renders pulmonary infection difficult to control. Pneumonia is an acute disease and tuberculosis a chronic one, but in neither case has specific therapy met with much success.

Attempts to work out a successful serum treatment in pneumonia date from the discovery that lobar pneumonia was a pneumococcal infection, but not until recently have any encouraging results been obtained. Undoubtedly, the development of a satisfactory specific therapy in pneumonia has been greatly retarded by insufficient knowledge of the biologic characteristics of the pneumococcus. It was only seventeen years ago that Neufeld and Händel [1] discovered that there was a definite difference between various pneumococci in their reactions to immune serum; in other words, that pneumococci could be grouped into various serologic types. The serum produced with organisms of one type did not protect against infection with other strains. In view of this specificity of types, these authors emphasized the importance of determining the type of pneumococcus in any individual case so that a corresponding immune serum might be used. They produced a potent antipneumococcus serum by immunizing horses with virulent pneumococci, and used it in the treatment of pneumonic patients by intravenous injections. Their results were encouraging. They called attention to the fact, since corroborated by others, that moderate amounts of serum had no beneficial effect and, for that reason, that large doses were indicated.

* The twelfth Mellon Lecture read before the Society of Biological Research of the University of Pittsburgh, May 12, 1927.

1. Neufeld and Händel: Arb. a. d. k. Gsndhtsamte. **34**:293, 1910.

The immunology of the pneumococcus was further cleared up by the work of Dochez and Gillespie,[2] and Dochez and Avery [3] in their studies on types of pneumococcus. These investigators studied the various types of pneumococcus in the United States, and found that there were three dominant biologic groups, each possessing specific and characteristic immune reactions. In their experience, these three types comprised about 80 per cent of all strains of pneumococcus encountered in patients with lobar pneumonia, and represented apparently fixed types of a highly parasitic nature. These three types were referred to as types I, II and III. The remaining 20 per cent of their patients had pneumococci which were, for the most part, unrelated biologically, and which, for convenience, they classified collectively as pneumococcus type IV. This is really an assembly of all other types of pneumococcus, many of them just as distinct as types I, II and III. At first, it was thought that each individual strain of pneumococcus type IV was immunologically distinct, but Olmstead [4] has shown that certain strains have many common characteristics, thus proving that there are definite subgroups in the type IV group of pneumococci. The type IV group is sometimes referred to as the "waste basket group" because it contains all pneumococci not contained in the three fixed types. Pneumococci of the type IV group are the organisms most frequently encountered in the secretions from the mouth of healthy persons. Dochez and his co-workers determined the incidence and death rate for the four so-called types of pneumococcus, and the more recent studies of Avery,[5] Stillman,[6] and others have thrown considerable light on the epidemiology of the disease with respect to the various types.

The discovery of the biologic types of pneumococcus made it clear why previous efforts to produce an effective serum against pneumonia had been unsuccessful. At the same time, these discoveries made it evident that the problem of producing a specific cure for pneumonia was destined to be much more difficult than it had been, for example, in the case of diphtheria and tetanus, in which the organism produced a highly specific toxin readily neutralized by antitoxin. It was now necessary to look on pneumonia as a group of biologically different infections, each of which required its own specific serum. However, the promising results obtained by Neufeld and Händel encouraged Cole and his associates at the Rockefeller Hospital to take up an extensive therapeutic investigation of the whole subject. A specific serum was produced in horses for both

2. Dochez, A. R., and Gillespie, L. J.: A Biologic Classification of Pneumococci by Means of Immunity Reactions, J. A. M. A. **61**:727 (Sept. 6) 1913.
3. Dochez, A. R., and Avery, O. T.: J. Exper. Med. **21**:114, 1915.
4. Olmstead, M.: J. Immunol. **2**:425, 1917.
5. Dochez, A. R., and Avery, O. T.: J. Exper. Med. **22**:105, 1915.
6. Stillman, E. G.: J. Exper. Med. **24**:651, 1916.

type I and type II pneumococcus, and patients with pneumonia of these types were treated with the homologous serum. The studies of Cole and his co-workers [7] mark the beginning of the modern specific therapy of pneumonia. Since the work of Cole, other investigators have taken up the problem, and much additional light has been thrown on the whole subject. I shall review briefly the work of these various investigators. Before taking up specific therapy proper, however, it might be well to recall a few facts pertaining to immunity to the pneumococcus, for it is only by an understanding of this question that one can appreciate the goal toward which immunologists are now working in the study of pneumonia.

IMMUNITY TO THE PNEUMOCOCCUS

It is not unusual for one person to have repeated attacks of pneumonia. For this reason, the idea has become prevalent that pneumococcic infection does not confer immunity. This, however, is not the case. Recurrent attacks of pneumonia in the same person are nearly always due to different strains of the pneumococcus, each producing, of course, its own specific immunity, but not conferring immunity against the other strains. Cecil and Blake [8] found that monkeys recently convalescent from an attack of pneumococcus type I pneumonia could not be reinfected with pneumococcus type I. They could be infected, however, with pneumococcus types II, III or IV. This specific immunity was also demonstrable after pneumonia caused by the other types. Dochez, Blake and, more recently, Baldwin and Rhoades, have pointed out that patients recovering from lobar pneumonia develop immune bodies against the pneumococcus at about the time of the crisis. The inference is that these antibodies play a significant part in immunity to the pneumococcus.

It is a comparatively easy matter to immunize animals against the pneumococcus. This immunity can be conferred by either active or passive immunization. As far back as 1910, Neufeld and Händel [9] showed that animals vaccinated with killed or living cultures of pneumococcus, developed an immunity against the organism. This fact has been amply verified by other investigators, more recently by Cecil and Steffen [10] who found that monkeys vaccinated against the pneumococcus were immune to pneumonia caused by a pneumococcus of the same type.

Chart 1 shows the variations in temperature and the number of white blood cells in type I by three subcutaneous injections of pneumococcus type I vaccine. Monkeys 13, 14 and 15 received the vaccine; monkey 21

7. Cole, R.: Treatment of Pneumonia by Means of Specific Serums, J. A. M. A. **61**:663 (Aug. 30) 1913.

8. Cecil, R. L., and Blake, J. A.: J. Exper. Med. **31**:685, 1920

9. Neufeld and Händel: Arb. a. d. k. Gsndhtsamte. **34**:293, 1910.

10. Cecil, R. L., and Steffen, G. I.: J. Exper. Med. **34**:245, 1921; **38**:149, 1923.

was an unvaccinated control. Three weeks after the completion of vaccination, an attempt was made to infect these monkeys by injecting them intratracheally with a living virulent culture of pneumococcus type I. The three vaccinated monkeys remained well; the control developed pneumococcus type I pneumonia associated with pneumococcus type I septicemia.

In 1917, Cecil and Austin [11] vaccinated 12,519 recruits against pneumonia at Camp Upton, using a mixed vaccine composed of equal parts of pneumococcus types I, II and II. These men were under observation

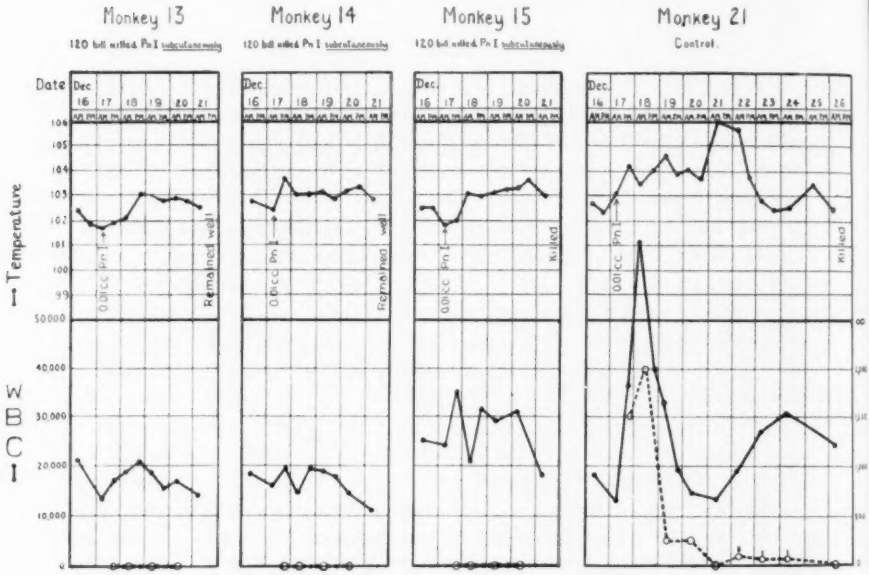

Chart 1.—Active immunization of monkeys against pneumococcus type I pneumonia following vaccination with pneumococcus type I vaccine.

for nearly three months subsequent to vaccination. During that time, only one case of pneumonia of the fixed type occurred among the vaccinated troops, and this patient had received only one inoculation of vaccine. Among the 19,481 unvaccinated troops, there were twenty-six cases of pneumonia of the fixed type during the same period of time. Furthermore, there was considerably less type IV pneumonia among the vaccinated troops than among the unvaccinated.

Active immunization, therefore, against the pneumococcus is feasible. It is also possible to confer a passive immunity on animals against

11. Cecil, R. L., and Austin, J. H.: J. Exper. Med. **28**:19, 1918.

pneumococcus infection, and this, of course, has considerable significance from the standpoint of serum therapy. If the serum from an animal immunized against pneumococcus type I is injected into a mouse, the mouse is immediately protected against pneumococcus type I infection.

The same is true of monkeys. In chart 2 are shown the variations in temperature and the number of white blood cells of three monkeys that

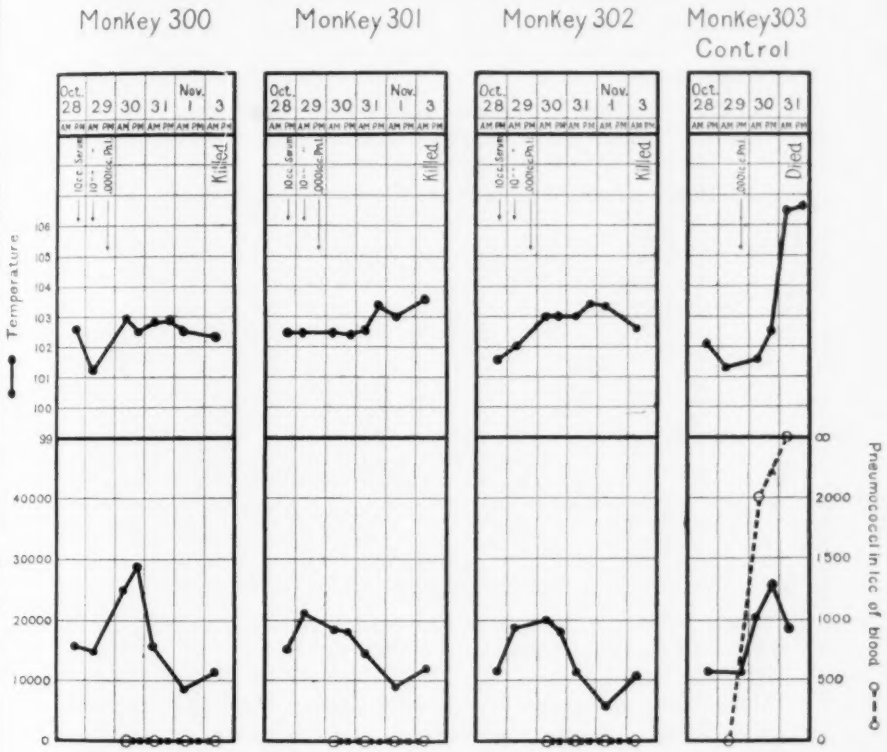

Chart 2.—Passive immunization of monkeys against pneumococcus type I with type I serum.

were passively immunized against pneumococcus type I. Monkeys 300, 301 and 302 each received two intravenous injections of type I anti-pneumococcus serum. Twenty-four hours later, an attempt was made to infect these monkeys by intratracheal injection of a living virulent culture of pneumococcus type I. All three monkeys remained well. The control monkey, number 303, had not received any serum, and the intratracheal injection of a virulent culture of pneumococci produced a fulminating pneumonia, septicemia and death.

Immunity to the pneumococcus is probably dependent on a number of factors. The humoral factors that are known are agglutinins, precipitins, opsonins and the so-called protective bodies. The protective bodies receive their name from the fact that when mice are injected with virulent cultures of pneumococci, they are protected from death by the simultaneous injection of a certain amount of antipneumococcus serum. None of these immune bodies has been isolated in a chemically pure form, but they can all be removed from serum by various chemical procedures which will be discussed later.

The protective bodies, like the other immune bodies, are highly specific for type, and are usually demonstrable in the serum of patients recovering from pneumonia at about the time of the crisis. Sometimes,

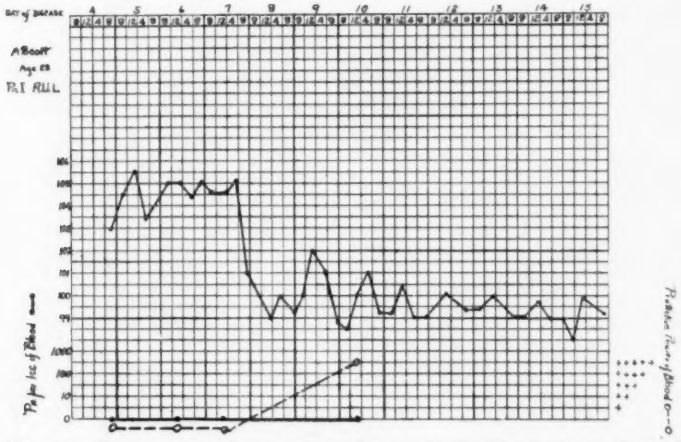

Chart 3.—Development of protective substance in the blood of a patient with pneumococcus type I pneumonia after the crisis.

however, they are present several days before the crisis. Chart 3 shows the development in a patient with type I pneumonia of a protective substance in the circulating blood after the crisis. The patient had his crisis on the morning of the eighth day. Up to this time, no protective substance had been present in the circulating blood. On the tenth day, the blood was again tested on mice, and enough protective substance was present to protect mice against 10,000 fatal doses of a culture of pneumococcus type I.

In addition to the humoral factors in immunity to the pneumococcus, there are important cellular factors. The leukocytes, notably the polymorphonuclear type, play an important rôle in pneumococcus immunity, and there is considerable evidence to show that the epithelial cells lining the terminal alveoli in the lung are vitally concerned.

One of the most interesting of recent developments in the field of immunity to the pneumococcus has to do with the so-called soluble substance. Physicians are chiefly indebted for their knowledge of this interesting product to Avery and his co-workers. The soluble substance, or S. substance as it is frequently called, is a polysaccharid which is always present in solution in broth filtrates of virulent cultures of pneumococcus. It can also be demonstrated in empyema fluids of pneumococcal origin and in the blood of patients with pneumococcus septicemia. It is usually present in the urine of patients with severe pneumonia. The soluble substance, like the protective substance, is rigidly specific for each type of pneumococcus. It is nontoxic and nonantigenic, but it has the important faculty of neutralizing the immune bodies produced by the patient. The soluble substance originates in the capsule of the pneumococcus and appears to enhance the virulence of the organism chiefly by preventing its phagocytosis by the leukocytes.

There has been considerable discussion recently as to whether the pneumococcus is capable of producing a toxin. Certainly, the pneumococcus has not been shown to produce a soluble toxin in the commonly acknowledged sense. Cole [12] showed that when solutions of pneumococci, obtained by dissolving them in bile salts, were injected intravenously into guinea-pigs, they produced sudden death similar to that seen in acute anaphylaxis. Cole admitted, however, that there was considerable doubt whether the substance producing this effect was identical with or related to the substance giving rise to the lesions and symptoms associated with pneumococcus infection. Solutions containing dissolved pneumococci have been found to be hemolytic, and there is evidence that this hemolytic substance exists pre-formed in the bacterial cells.

Recently, Clowes, Jamieson and Olson [13] claimed to have demonstrated a toxic substance in the filtrates of cultures of pneumococcus. This toxin does not often kill laboratory animals, but when injected subcutaneously or intravenously, it is capable of producing hemorrhagic lesions in the lungs. Olson [14] claimed that the serum of animals, immunized against this so-called toxin, contains an antitoxin which when mixed with toxin will prevent the development of the hemorrhagic lesions in the lungs. This toxin also gives a positive reaction to skin test in rabbits, which can be neutralized by the antitoxin. These investigators said that the pneumonococcus toxin and antitoxin which they prepared apparently are not specific for type. On the basis of this work, Clowes, Jamieson and Olson have prepared "antitoxin" for therapeutic purposes, but, so far, this product has not received any extensive

12. Cole, R.: J. Exper. Med. **20**:346, 1914.

13. Clowes, G. H. A.; Jamieson, W. A., and Olson, J. G.: Proc. Soc. Exper. Biol. & Med. **23**:334, 1925-1926.

14. Olson, J. G.: Proc. Soc. Exper. Biol. & Med. **23**:331, 1925-1926.

clinical test. My co-workers and I have treated a few patients at Bellevue Hospital with this antitoxin, but the results have not been impressive.

CAUSE OF DEATH IN PNEUMONIA

Before proceeding to an actual discussion of the specific treatment of patients with pneumonia, I shall discuss briefly the cause of death in this disease. In city hospitals, about 30 per cent of all patients with lobar pneumonia die. There are slight variations in this figure for different hospitals and different localities, but any large group of carefully selected cases of lobar pneumonia will show a death rate close to this figure. It is easy to understand why a considerable number of these patients die; they are old or they are suffering from some chronic systemic disease which has already undermined their resistance to infection. But the question which interests one particularly is: Why is pneumococcus infection fatal in a certain percentage of healthy young adults?

TABLE 1.—*Death Rate in Pneumonia for the Various Types*

	Cases	Died	Per Cent
Type I	352	73	20.7
Type II	221	93	42.0
Type III	161	67	41.6
Type IV	373	109	29.2
Total	1,107	342	30.8

The death rate of pneumonia varies considerably with the type. In 2,000 cases of typed pneumococcus pneumonia, recently analyzed by Cecil, Baldwin and Larsen,[15] the death rate for patients that received no specific therapy is shown in table 1.

It will be seen from these figures that while types II and III pneumonia are the severest forms, types I and IV actually kill more patients because they are so much commoner than the other two types. Type I pneumonia has the lowest death rate of all the four types, presumably because it is essentially the pneumonia of young people.

For many years, physicians have assumed that patients with pneumonia die of heart failure, but if this is true, it is not heart failure in the ordinary sense. There is considerable evidence to show that in many instances the patient with pneumonia dies of vasomotor paralysis. In fatal cases, the blood pressure is usually extremely low for several

15. Cecil, R. L.; Baldwin, H. S., and Larsen, N. P.: Lobar Pneumonia: A Clinical and Bacteriologic Study of Two Thousand Typed Cases, Arch. Int. Med. **40**:253 (Sept.) 1927.

hours or even days before death occurs. But one may go back a step further and ask the reason for the heart failure or the vasomotor paralysis.

There is one fact the significance of which all students of pneumonia are agreed on, namely, that fatal pneumonia is usually a septic pneumonia. Patients dying with pneumococcus pneumonia nearly always show a considerable number of pneumococci in the circulating blood. In this respect, pneumonia is like most other pyogenic infections. Starting out as a local process in the lung, it soon produces symptoms of toxemia, but the life of the patient is rarely in danger as long as the blood stream remains sterile. With the development of septicemia, the situation becomes much more serious. In Cole's studies, 343 cases of lobar pneumonia in which blood cultures were negative showed a death rate of only 11.6 per cent; while in 119 cases in which the blood cultures were positive, the death rate was 67.1 per cent. Moreover, the death

TABLE 2.—*Blood Cultures in Case of Lobar Pneumonia of Various Types*

Type of Pneumococcus	Cases Studied	Cases Showing Positive Blood Cultures		Cases Showing Sterile Blood Cultures	
		Cases	Deaths	Cases	Deaths
I	39	11	6	28	3
II	22	14	13	8	1
III	11	4	3	7	1
IV	35	8	7	27	2
Total	107	37	29 78.3%	70	7 10%

rate was directly proportional to the number of pneumococci present in the blood. In patients who showed over twenty colonies per cubic centimeter of blood, the death rate varied from 80 to 100 per cent. Similar observations were obtained in our studies of pneumococcus septicemia at Bellevue Hospital. In 107 cases of lobar pneumonia of various types, frequent blood cultures were taken throughout the course of the disease.

In table 2 the relation of bacteremia to the death rate is clearly shown. In seventy cases with sterile blood culture, the death rate was only 10 per cent; whereas, in thirty-seven cases with positive blood cultures the death rate was 78.3 per cent. Therefore, while one is hardly in a position to say that death in pneumonia is actually due to pneumococcus septicemia, one can at least say that death in pneumonia is associated in a high percentage of cases with pneumococcus septicemia and that pneumococcus septicemia is usually followed by death. In studying experimental pneumococcus pneumonia in several hundred monkeys, I have seen a great many of these animals die of the disease, but not a single case that I can recall in which death was not accompanied by a large number of pneumococci in the blood.

SIGNIFICANCE OF IMMUNE BODIES IN PNEUMOCOCCUS PNEUMONIA

I have already referred to the fact that recovery in pneumonia is accompanied by the development of various immune bodies in the blood of the patient. Agglutinins, precipitins, complement-fixing bodies and protective bodies make their appearance at about the time of the crisis and persist in the blood of the convalescent patient for several weeks or even months after recovery. So regularly does this occur that physicians have learned to be fearful about the outcome of the disease in

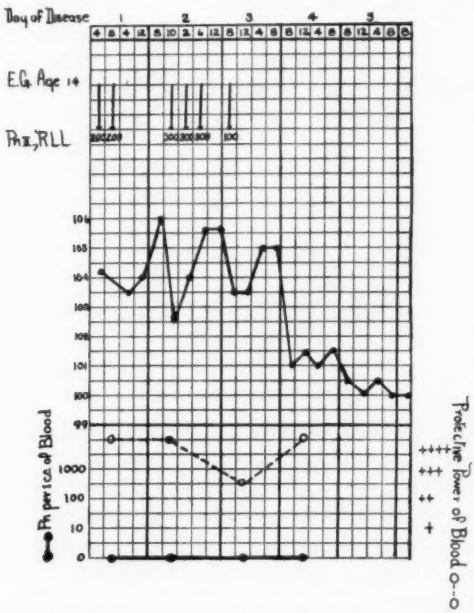

Chart 4.—Variation in temperature of a patient with pneumococcus type II pneumonia. Immune bodies were in the blood on the first day of the disease.

any patient if the blood shows a complete absence of immune bodies, even when the temperture, pulse and respiration have returned to normal. Sometimes, the immune bodies appear in the blood two or three days before the crisis; this, of course, is a good omen. In one case of type II pneumonia which my co-workers and I studied in a boy, aged 15, a large amount of protective substance was found in the circulating blood on the first day of his disease. The infection ran a short course (chart 4), the temperature becoming normal on the fifth day. But there were two other significant factors in this case. The patient gave

little evidence of toxemia. He wanted to sit up in bed and read even when the temperature was 105 F., and there was never any delirium. The second significant factor was that pneumococci did not appear in the blood at any time. The latter point is particularly significant. In a series of twenty-five cases of pneumonia studied in our laboratory, in which blood was taken every day or two to determine the presence or absence of pneumococci and of protective bodies, bacteria and specific antibodies occurred simultaneously in the blood only once, and in that instance the blood showed only three or four pneumococci per cubic centimeter of blood. From this it may be inferred that one of the most important functions of immune bodies is to prevent the entrance of pneumococci into the general circulation. On the other hand, patients

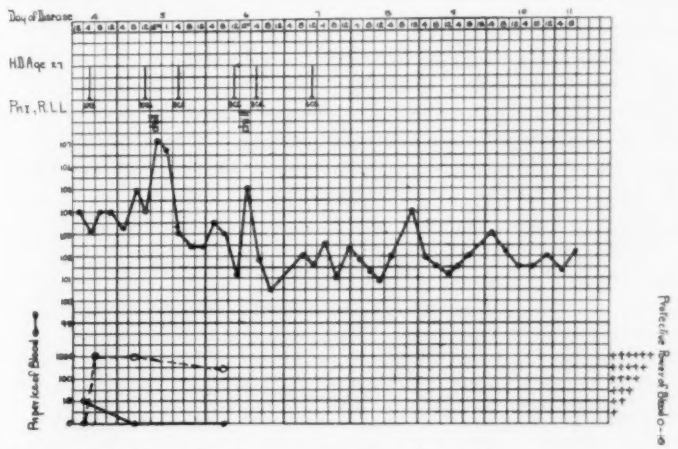

Chart 5.—Variations in temperature of a patient with pneumococcus type I pneumonia with positive blood culture. Empyema developed in spite of immune bodies in patient's blood.

who die of pneumonia rarely show immune bodies in the circulating blood, but, as I have already shown, they usually do show a severe grade of pneumococcic sepsis. In some of these fatal cases, the blood contains so many pneumococci during the last hours of life that it is almost impossible to count the colonies on a plate. In the blood and urine of these septic patients a large amount of the soluble substance of Avery can be demonstrated. As this substance has the faculty of neutralizing the immune bodies, one may infer that whatever immune substance has been manufactured by the patients has been completely neutralized by the soluble substance produced by the pneumococci in the circulating blood. While the protective antibodies appear to shield the patient against pneumococcus sepsis, they do not necessarily prevent

certain other pneumococcus complications. For example, chart 5 shows the temperature curve of a patient with pneumococcus type I pneumonia in which the crisis was accompanied by the development of protective antibodies in the patient's blood. A few days later, this patient developed empyema. Examination of the blood showed that the patient's serum still contained a considerable amount of immune bodies in spite of the empyema. The significant point is that the blood remained sterile and the patient eventually recovered.

What is the exact function of the protective antibodies? The studies of Blake,[16] and the more recent work of Robertson and Sia [17] show that the pneumococcus immune bodies are necessary for the phagocytosis of virulent pneumococci, in this respect resembling Wright's opsonins. Indeed, the protective antibody and opsonin may be identical substances. Virulent pneumococci will grow even in the most highly potent anti-pneumococcus serum, but under such conditions the pneumococcus rapidly loses its virulence. Years ago, Neufeld and Händel showed that the leukocytes do not take up virulent pneumococci, while avirulent strains undergo rapid phagocytosis, when virulent pneumococci are mixed with normal human blood and allowed to stand in the incubator for twenty-four hours, the pneumococcus grows freely. In the blood of a patient convalescing from pneumonia, the pneumococcus usually fails to grow under the same circumstances. Furthermore, if immune serum is added to the blood of a normal man, the pneumococcus will not grow. However, if the blood of the convalescent patient is first centrifugalized and the leukocyte cream removed, the pneumococcus grows freely. From this evidence, it appears almost certain that the final destruction of pneumococci is accomplished by the leukocytes, but that they will not undertake this important function until the pneumococci have been prepared by the serum for phagocytosis.

Just as the protective bodies are conducive to phagocytosis, so the soluble substance interferes with phagocytosis by blocking the immune bodies and thereby allowing the pneumococci to maintain their full virulence.

In pneumococcus infection in man, the ultimate outcome depends on whether the pneumococci or the leukocytes gain supremacy. If the host produces enough immune bodies, the infection remains localized in the lung, and the patient recovers. On the other hand, if the pneumococci produce enough soluble substance to more than neutralize the immune bodies, phagocytosis is inhibited, the infection spreads, the pneumococci gain access to the blood stream and the patient eventually dies with pneumonia sepsis.

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16. Blake, F. G.: J. Exper. Med. **26**:563, 1917.

17. Robertson, O. H., and Sia, R. H. P.: J. Exper. Med. **39**:219 (Feb.) 1924; **40**:467 (Oct.) 1924.

THERAPEUTIC INTRODUCTION OF PROTECTIVE SUBSTANCE INTO THE BLOOD

In the fight then between the pneumococcus and the human host, septicemia spells death and immune bodies recovery for the patient. Herein lies the rationale of our efforts to produce a specific therapy for lobar pneumonia. The modern serum treatment of patients with pneumonia consists essentially in the artificial introduction of pneumococcus immune bodies with the hope of assisting the patient in his efforts to produce immunity against the disease.

The artificial immunity which one tries to establish in the pneumonic patient is probably antibacterial. By the introduction of an excess of protective antibodies one hopes to sensitize or opsonize the pneumococci and thus prepare them for phagocytosis by the leukocytes. So far as is known, there is no genuine antitoxic quality in any pneumococcus serum.

It should be emphasized here that the various specific therapeutic agents are essentially alike in their general nature in that they all contain protective antibodies and usually agglutinins and precipitins. Up to the present time, three of these specific therapeutic agents have been studied in our clinic and laboratory at Bellevue Hospital; (1) the type I and type II antipneumococcus serum of Cole; (2) Huntoon's pneumococcus antibody solution and (3) Felton's concentrated antipneumococcus serum.

TYPE I ANTIPNEUMOCOCCUS SERUM

It must be evident from what has already been said regarding the specificity of pneumococcus types that the efforts of early investigators to produce efficient antipneumococcus serum were frustrated by their failure to distinguish between these various groups of pneumococci.

Following the work of Neufeld and his co-workers, Rufus Cole took up the question of specific therapy at the Hospital of the Rockefeller Institute. Working first with pneumococcus type I, Cole's type I serum was prepared as follows:

> Horses were immunized by injections first of dead and then of living cultures of type I pneumococci. The serum of the horse was tested for its power to produce agglutination of pneumococci of this type, and also for its effectiveness in protecting mice against multiple lethal doses of the same organism. Cole used the so-called "mouse protection test" to determine the therapeutic power of type I serum. The standard which he finally set for an accepted therapeutic serum was one in which the serum should be of such a strength that 0.2 cc. would protect mice regularly against at least 0.1 cc. of a pneumococcus type I culture of such virulence that 0.0000001 cc. of an eighteen hour broth culture would kill a mouse within forty-eight hours. When the horse had produced a serum of such potency, it was bled under aseptic conditions, and after the blood had clotted, the serum was removed and placed in bottles for use.

Cole advocates the use of type I serum for only those patients whose sputum or blood have been found to contain pneumococcus type I. This preliminary examination causes some loss of time; but Cole claims, and I think rightly, that patients with other types of pneumonia should not be subjected to type I serum.

In type I cases, the serum is administered as follows:

In order to avoid anaphylactic accidents, the patient is first questioned for a history of previous injections of horse serum and also for a history of hay-fever or asthma, as persons having such symptoms are likely to be sensitive to various proteins including those in serum. In any event, an intradermal skin test is performed with a small tuberculin syringe, 0.02 cc. of diluted horse serum being injected into the skin. If the reaction to the test is negative, the wheal produced by the injection of serum fades away rapidly. If sensitiveness does exist, a genuine urticarial wheal begins to develop, usually within five minutes, which may increase slowly in size up to that of a half dollar. This lesion usually reaches its maximum within an hour and then fades away rapidly.

Cole believes that all patients who are to receive antipneumococcus serum should receive a "desensitizing" dose of serum six or eight hours before the first intravenous injection. The reason for this is that occasionally one sees a patient in whom the reaction to the skin test is negative and yet in whom, on the injection of large amounts of serum, symptoms of serum sensitiveness appear. It is therefore advisable to inject 1 cc. of antipneumococcus serum subcutaneously even though the reaction to the skin test is negative.

In the small percentage of patients who are found to be sensitive to horse serum, a more thorough method for desensitization must be employed. This consists in giving small amounts of serum subcutaneously at half hour intervals, doubling the size of the dose at each injection. After 25 cc. of serum have been given by this method, larger doses, consisting of from 50 to 100 cc. may be injected intravenously without fear of shock.

Type I serum is administered intravenously. The serum is usually allowed to run into the vein by gravity and should be injected slowly. Cole advises that the injection of the first 10 to 15 cc. of serum should occupy from ten to fifteen minutes. During this period, one watches carefully for any change in the patient's appearance and for increased rapidity of the pulse, dyspnea, cyanosis or urticaria. If these symptoms appear, it is well to stop the injection of serum for a few minutes to see if the symptoms increase in severity. Usually, they disappear rapidly and the treatment can be resumed. Experience has shown that if antipneumococcus serum is to be successful, it must be given in large doses. It is necessary to inject a sufficient amount of serum to produce a balance of protective antibodies in the patient's blood, such as that which occurs during natural recovery from the disease; for, as already indicated, it is largely on this factor that recovery depends. An effective concentration of immune bodies can be obtained in the blood only after all the circulating soluble substance has been neutralized. Once a permanent balance of antibodies in the blood has been achieved, a fall in temperature and a noticeable improvement in the condition of the patient should soon follow. The amount of serum necessary will vary in each case. Cole recommends an initial dose of from 90 to 100 cc. The frequency and size of the succeeding doses must be regulated largely by the effects obtained by those preceding, but in most cases the serum should be given every eight hours in doses of from 90 to 100 cc. until the patient's temperature drops

to 100 F. or less and remains at that point. The patient should be under observation day and night and the treatment should be given by night as well as by day whenever necessary.

REACTIONS TO SERUM

Cole describes three types of reaction which may follow the injection of antipneumococcus serum: (1) the true anaphylactic reaction, (2) the thermal reaction and (3) the serum disease.

In persons who are sensitive to horse serum a more or less severe asthmatic attack with dyspnea and flushing of the face may develop at once or within from fifteen to twenty minutes after the introduction of serum, followed by cyanosis, sweating, cough, anxiety and an urticarial rash. An anaphylactic reaction, unless extremely severe, is usually relieved by a hypodermic injection of from 0.5 cc. to 1 cc. of a 1:1,000 dilution of epinephrine. These reactions rarely occur, and if sensitive patients are properly desensitized, serious anaphylactic reactions will probably never occur.

Another type of reaction which may follow the intravenous injection of serum is the so-called thermal or foreign protein reaction. This comes on usually from thirty minutes to one hour after injection and is characterized by a chill, some cyanosis, rapid rise in temperature of from 1 to 3 degrees, which is followed by an equally rapid fall to normal or even to subnormal. During the fall there is usually profuse perspiration. After the reaction, the patient usually feels much improved. Sometimes the temperature remains normal, but usually it begins to rise again after a few hours. Cole does not believe that this reaction is of benefit to the patient and thinks it should be avoided if possible. Patients with this type of reaction need little treatment other than reassurance and the application of heat to the extremities.

Following the administration of horse serum, a group of symptoms frequently occurs which are referred to as serum sickness or serum disease. These symptoms usually make their appearance from seven to fourteen days following the administration of serum and consist of fever, skin rashes, most frequently urticaria or erythema, edema of the skin, general adenopathy and pain and swelling in the joints. The attack usually lasts from a few days to a week and may recur one or more times at intervals of a few days. Not all patients receiving serum show the symptoms. According to Cole, mild symptoms of serum disease appear in about one-half of the treated patients; severe attacks, rarely. The severe cases are more likely to occur in patients who receive large doses of serum.

RESULTS OF SERUM TREATMENT

The most striking effects of serum treatment in type I pneumonia are usually seen after a so-called thermal reaction. Sometimes the

clinical change after one of these shock reactions is spectacular, and the marked amelioration of symptoms presents the features of a natural crisis. In cases in which a thermal reaction does not occur, a change in the patient's condition is usually not so marked. Even when chill is absent, the patient's mental condition is generally improved, cyanosis becomes somewhat less, and the pulse rate falls. No doubt, in many cases this improvement is due to an actual sterilization of the blood. Cole thinks that, in addition to its other effects, antipneumococcus serum may have a detoxifying effect.

In our experience, the most striking results in type I therapy have been:

1. When serum was administered on the first or second day of the disease and the whole infection was aborted, the temperature dropped rapidly to normal. In such cases, the physical signs never become frank, and the patient is practically well in forty-eight to seventy-two hours.

2. Type I serum produces a marked improvement in septic patients whose blood contains 50 or 100 colonies of pneumococcus type I per cubic centimeter by sterilizing the blood and thereby reducing the infection to a localized process. Cole believes that the administration of serum does not cause any change in the rate of resolution of the lung tissue already involved. When given early, however, there is no doubt that the serum prevents spread of the infection to healthy tissue. There is no evidence that serum diminishes the incidence of pneumococcic complications.

EFFECT OF SERUM ON MORTALITY RATE

In Cole's series of 195 patients with the pneumococcus type of pneumonia that were treated with serum, there was a mortality rate of only 9.2 per cent. In type I cases, before serum treatment was commenced, the death rate had been from 25 to 30 per cent. According to these figures, therefore, the death rate for patients with type I pneumonia had been reduced two-thirds by the use of serum.

Since the original report of Cole, the largest group of cases of type I pneumonia in which the patients have been treated with antipneumococcus serum is that of Wadsworth [18] who, in 1924 reported the cases of 445 in New York state treated with a highly potent type I serum prepared by the New York State Department of Health. When the cases studied in army camps were eliminated, there were 277 cases with fifty deaths, a death rate of 18 per cent. In 344 patients with type I pneumonia that had not received serum the death rate was only 19 per cent, a figure only slightly higher than that for the treated patients.

18. Wadsworth, A. B.: Am. J. Hyg. **4**:119 (March) 1924.

Shortly after Wadsworth's article was published, Locke[19] reported a detailed study of 145 cases of type I pneumonia in which the patients were treated with serum, with a mortality rate of 17.2 per cent as compared with a mortality of 16.9 per cent in 70 untreated patients.

After reading the excellent studies of Wadsworth and of Locke, one wonders why the results reported in their series of cases were so much less promising than those obtained by Cole. Wadsworth's patients were treated with a potent serum but by many different physicians, some of whom probably did not administer serum in sufficiently large doses. Locke's patients received plenty of serum, but he does not give any record of its potency.

THE EFFECT OF TYPE I SERUM IN EXPERIMENTAL TYPE I PNEUMONIA

It is not surprising that, in view of the results obtained by Wadsworth and by Locke, the popularity of type I serum has decreased considerably since its original introduction by Cole, and yet the low death rate obtained by Cole (9 per cent) is significant; furthermore, the results obtained in the treatment of monkeys that had experimental type I pneumonia with type I serum are most convincing. Cecil and Blake found that type I antipneumococcus serum has a highly specific therapeutic action on monkeys with experimental pneumococcus type I pneumonia.

Chart 6 shows the variations in the temperature of two monkeys with experimental pneumococcus type I pneumonia. Twenty-four hours after injection with a lethal dose of a culture of pneumococcus type I, a course of small intravenous injections of type I serum was started in monkey 94. The first injection sterilized the blood, and the temperature was reduced to 102 F. after five injections. Treatment was then stopped, but the temperature rose again on the fifth day. Two more injections of serum given at different times produced a crisis, and the monkey remained well thereafter. The control, money 93, did not receve serum and ran a typical course with septicemia, dying on the twelfth day.

Chart 7 shows the effect of type I serum on monkeys with experimental type I pneumonia when serum treatment was started late in the disease. These charts are instructive in that they show that when treatment with serum is started late, the serum is still capable of sterilizing the blood, but that it exerts practically no influence on the temperature. The lives of both treated monkeys (99 and 113) were saved, while the control monkey (112), in this instance treated with normal horse serum, died on the sixth day of the disease with lobar pneumonia and pneumococcus septicemia. Chart 8 is in sharp contrast to the

19. Locke, E. A.: Treatment of Type I Pneumococcus Lobar Pneumonia with Specific Serum, J. A. M. A. **80**:1507 (May 26) 1923.

preceding experiment showing the striking effect of serum when administered in large doses early in the disease. Both monkeys were given lethal doses of pneumococcus type I culture, and both recovered promptly after receiving four doses of serum.

To my mind, the three experiments just presented prove the therapeutic value of type I serum more convincingly than any number of statistics.

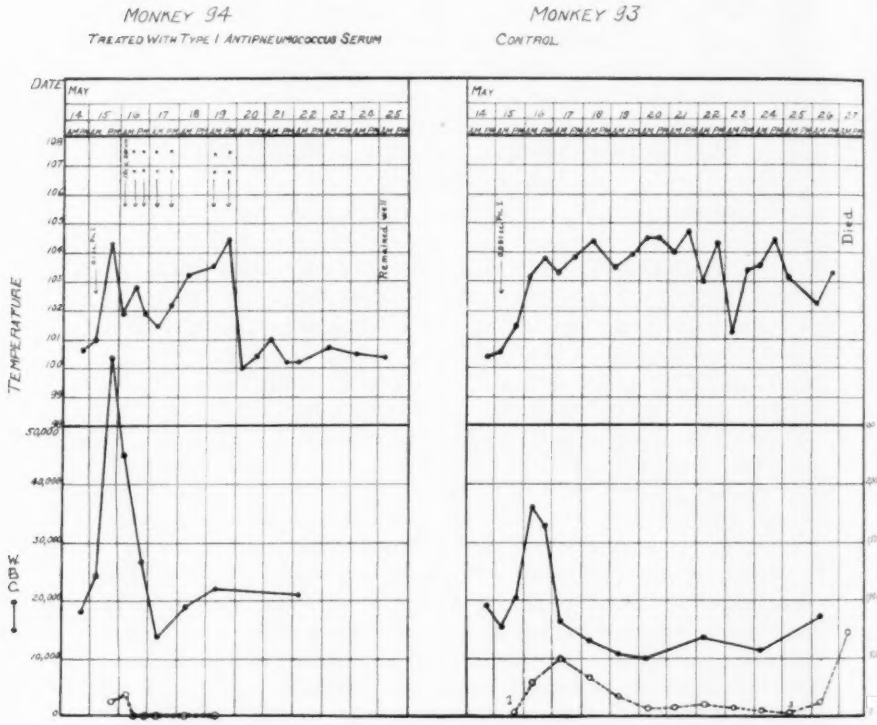


Chart 6.—Treatment of monkey with pneumococcus type I pneumonia with type I antipneumococcus serum.

Why has type I serum not come into more general use in the treatment of patients with type I pneumonia? Perhaps the most important obstacle to its use has been the difficulty in getting an early bacteriologic diagnosis. In many cases sputum is not obtainable until several days after the onset of the disease, and then too often at least twenty-four hours elapses before the laboratory makes its report. This means that treatment with serum is begun late in the disease when its therapeutic effect is not as striking as when it is administered early. Another objection to the gen-

eral use of type I serum is the fear of reactions, either anaphylactic or thermal. Another reason for its lack of popularity is the technical difficulty connected with the proper administration of large doses of serum. Cole advises that the serum be diluted with an equal amount of saline solution, and this means that three times in every twenty-four hours the busy practitioner must inject 200 cc. of diluted serum intravenously. Finally, a most important obstacle to its further use is the fact that many observers have not been able to duplicate the low mortality figures obtained at the Rockefeller Hospital. The reasons for this failure

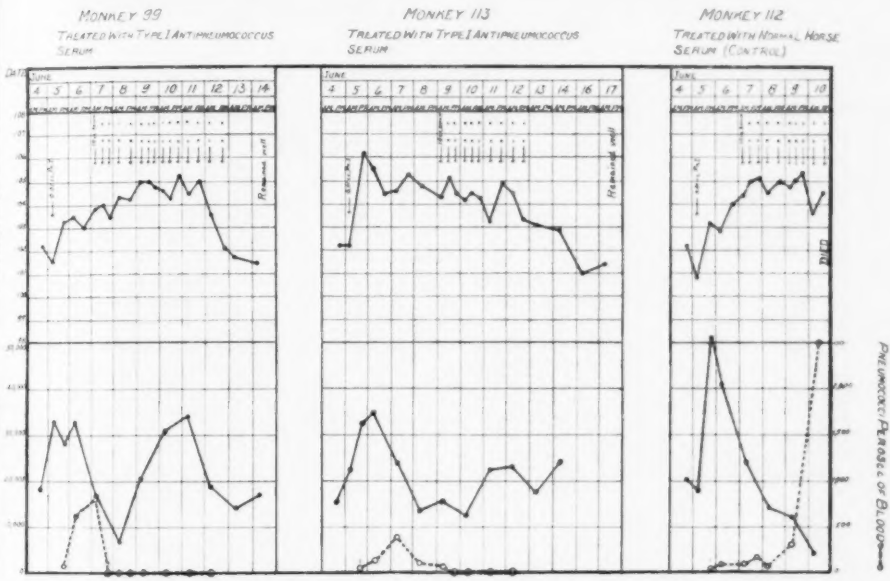


Chart 7.—Pneumococcus type I pneumonia in monkeys. Two monkeys (99 and 113) were treated with type I serum and recovered. The control, monkey 112, received normal horse serum and died.

may be several: (1) patients are not treated early enough; (2) the serum may not be sufficiently potent; (3) the serum is given in too small doses, or (4) the serum is not given frequently enough.

Before passing on to a discussion of derivations of serum something should be said about type II antipneumococcus serum. Theoretically, type II serum should be just as effective as that for type I, but in addition to the practical defects encountered in the use of type I serum, other problems are encountered in pneumococcus type II pneumonia. In the first place, it is almost impossible to develop a serum equal in potency

to that of a good type I serum; in the second place, pneumococcus type II when growing in the body of an animal or man produces much more specific soluble substance than pneumococcus type I. In view of the ability of this substance to neutralize homologous serum, it is obvious that type II infections will require a great many more antibodies than type I infections.

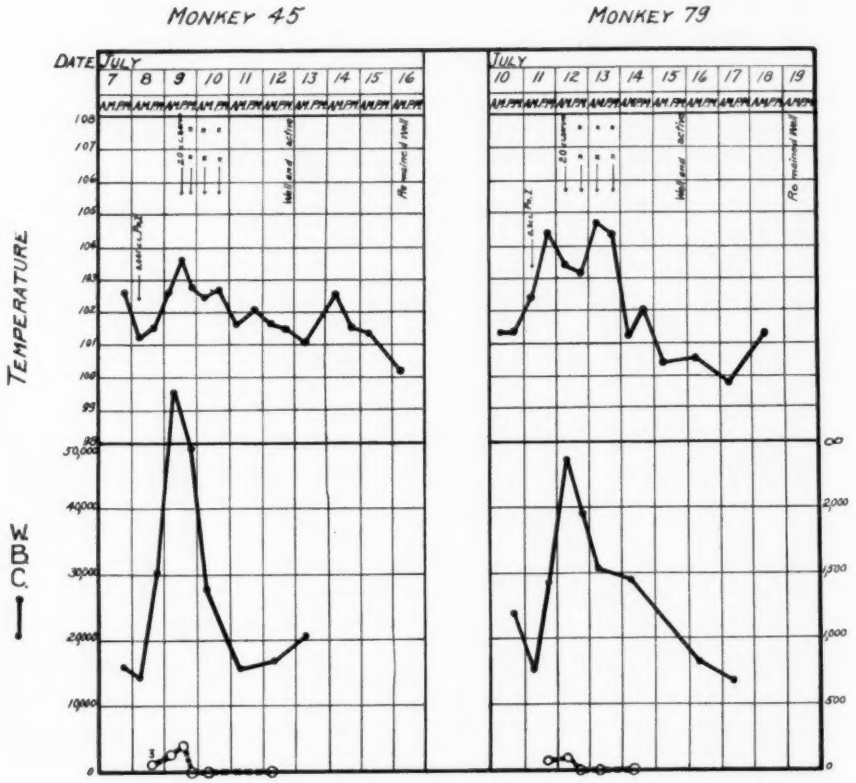


Chart 8.—Abortive type I pneumonia in monkeys produced by intensive treatment with type I serum.

Cole and his co-workers at the Rockefeller Hospital tried type II antipneumococcus serum on patients with type II pneumonia and were unable to demontrate any therapeutic effect on the course of the disease. During the winter of 1926, however, Dr. William H. Park of the New York City Board of Health immunized a horse against pneumococcus type II and obtained a serum of such potency that 0.2 cc. would protect a mouse against 0.2 cc. of pneumococcus type II culture; in other

words, the potency of this serum was equivalent to that of the best type I antipneumococcus serum.

Last winter my co-workers and I had an opportunity to test this serum on a number of patients with type II pneumonia at Bellevue Hospital. Chart 9 shows the variations in temperature of a patient with type II pneumonia treated on the third day of the disease with type II antipneumococcus serum. This patient had a sterile blood culture. One injection of serum, however, was sufficient to establish a reserve of immune bodies

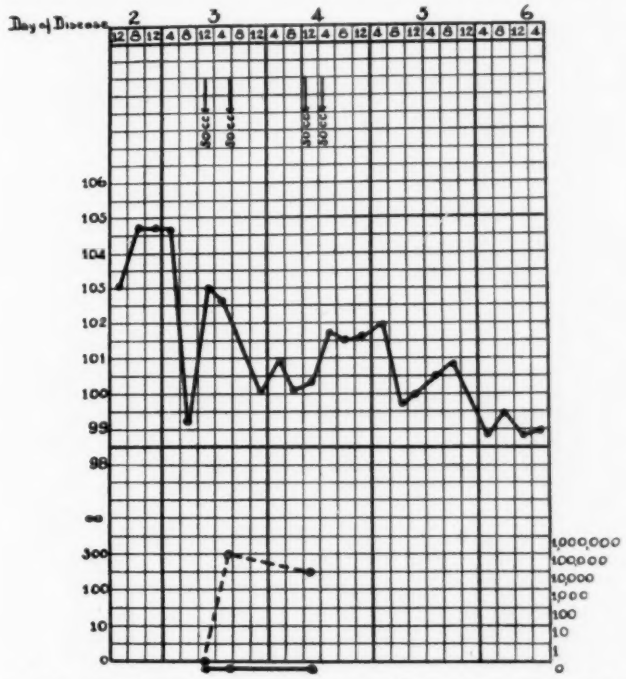


Chart 9.—Variations in temperature of a patient with pneumococcus type II pneumonia treated with type II antipneumococcus serum.

in the patient's blood. The patient received four other injections on the same day and two injections on the following day. The temperature came down at the beginning of the fifth day.

Chart 10 shows the variations in temperature in an interesting case of type II infection which was characterized by heavy septicemia on the second day. Two injections of serum brought about a complete sterilization of the blood, and after three more injections on the following day a balance of specific antibodies made their appearance in the blood and proceeded until the temperature reached normal.

Examples of this kind lead us to feel that in a certain number of patients with type II pneumonia in which treatment is begun early, the type II serum exercised a definite therapeutic effect. Unfortunately, the number of patients treated was not sufficiently large to furnish reliable statistical evidence.

In view of these obvious difficulties in the general use of antipneumococcus serum, it was natural that immunologists should make efforts to concentrate and purify type I serum. Gay and Chickering [20] were the first to show that the immune bodies in pneumococcus serum could be removed by chemical methods. By mixing a solution of pneumococcus

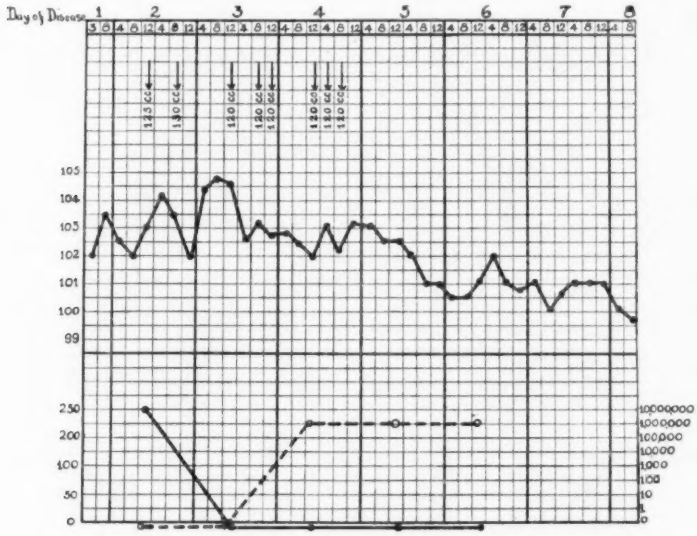


Chart 10.—Variations in temperature of a patient with pneumococcus type II pneumonia treated with type II antipneumococcus serum. Heavy septicemia apparently controlled by serum.

bodies and homologous antiserum, a voluminous precipitate resulted which contained practically all the immune substances of the serum. The immune bodies contained in this precipitate were then extracted in a diluted alkaline solution at 42 C. The resulting water-clear extract possessed the power to protect animals against pneumococcus infection, and it also contained other demonstrable antibodies, such as agglutinins and precipitins. By this method, a large proportion of the antibodies could be concentrated in a volume from one-fifth to one-tenth that of

20. Chickering, H. T., and Gay, F. P.: J. Exper. Med. **21**:389, 1915.

the original serum, but the product thus obtained was not stable, and the technic involved was so laborious that the authors considered the method impracticable for therapeutic purposes.

HUNTOON'S PNEUMOCOCCUS ANTIBODY SOLUTION

In 1921, Huntoon [21] published a series of articles which had to do with the removal of pneumococcus antibodies from antipneumococcus serum. The method used by Huntoon was somewhat like that employed by Gay and Chickering, but in one respect fundamentally different. The precipitate obtained by Gay and Chickering was derived in great part from the serum proteins; hence, their solutions contained serum and gave serum reactions. Huntoon used only formed antigens which could be washed free from serum. This method eliminated to all practical purposes all the serum constituents with the exception of the immune bodies themselves. Huntoon also introduced another innovation into the specific treatment of patients with pneumonia. His horses were inoculated against pneumococcus types II and III, as well as against type I. The serum was, therefore, trivalent, containing immune bodies against the three fixed types of pneumococcus.

The actual method of preparation of Huntoon's antibody solution is as follows:

> Pneumococci of the three fixed types are exposed to the action of a large amount of an antipneumococci immune serum, multivalent for types I, II and III. The sensitized organisms are removed by centrifugalization, carrying the attached antibody with them. The bacteria are then washed free from serum by repeated changes of salt solution. Finally, they are emulsified in salt solution containing 0.25 per cent sodium bicarbonate and are heated to 55 C. for from thirty minutes to one hour. The emulsion is again centrifugalized, and the supernatant removed, chilled, recentrifugalized, and finally filtered through a filter candle.

Huntoon's antibody solution is a water clear product with a total nitrogen content of only 0.035 mg. per cubic centimeter. It gives a negative biuret reaction, however, although no serum proteins are demonstrable by chemical means, but they are detectable in a small amount by sensitization tests on guinea-pigs. Huntoon's solutions are stable when preserved at low temperatures.

Huntoon's antibody solution usually contains a high content of protective bodies against pneumococcus type I, 0.2 cc. usually protecting against 0.1 cc. of a virulent type I culture. Its protective power against types II and III is not so marked. In many lots, 0.2 cc. of antibody will protect against 0.01 cc. of type II culture, but even at this figure it is only one-tenth as potent for type II as for type I. Against type III, the potency is even lower, the best lots rarely protecting against more than

21. Huntoon, F. M.; Masucci, P., and Hannum, E.: J. Immunol. **6**:185, 1921.

0.001 cc. of virulent type III culture. It can readily be seen that, theoretically at least, Huntoon's solution possesses great advantages over the original antipneumococcus serum. Practically free from animal protein, it eliminated at once the danger of anaphylactic reactions and the discomforts of serum sickness. Furthermore, it contained antibodies against all three of the fixed types of pneumococcus and could be employed at once without waiting for a bacteriologic examination of the sputum.

In the first place, it seemed desirable to test the value of Huntoon's solution on experimental pneumonia in monkeys, just as we had previ-

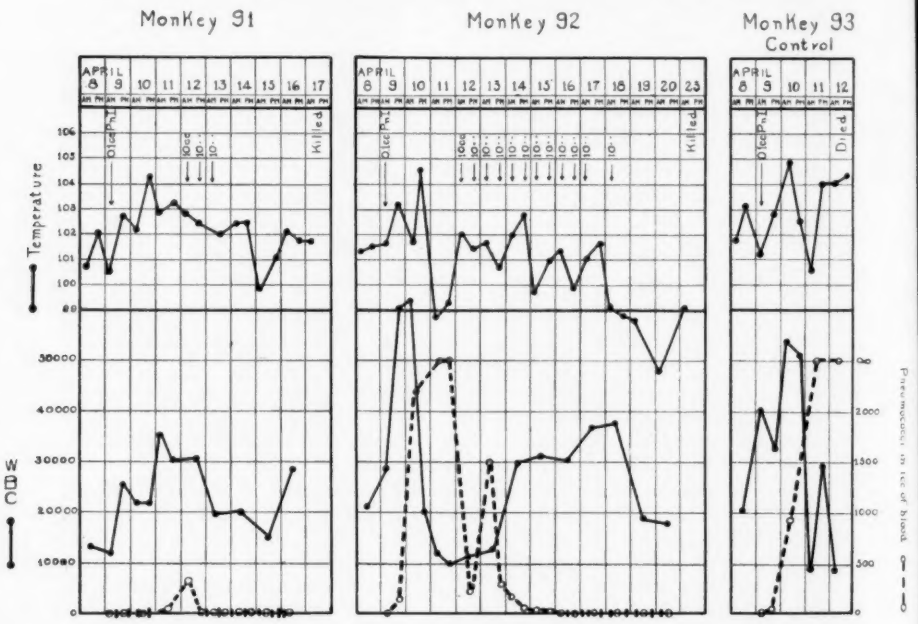


Chart 11.—Treatment of monkeys with pneumococcus type I pneumonia with Huntoon's antibody solution.

ously tested type I serum. Chart 11 shows the results for three monkeys subjected to experimental type I pneumonia. Each monkey received a lethal dose of type I culture intratracheally, and all promptly developed symptoms and signs of pneumonia and septicemia. Seventy-two hours after infection, monkeys 91 and 92 received their first injection of Huntoon's antibody solution. The treatments were continued twice a day as long as either monkey showed symptoms. It will be seen from the charts that in both instances the injections quickly drove the pneumococci from the blood and, following this, effected a rapid recovery in

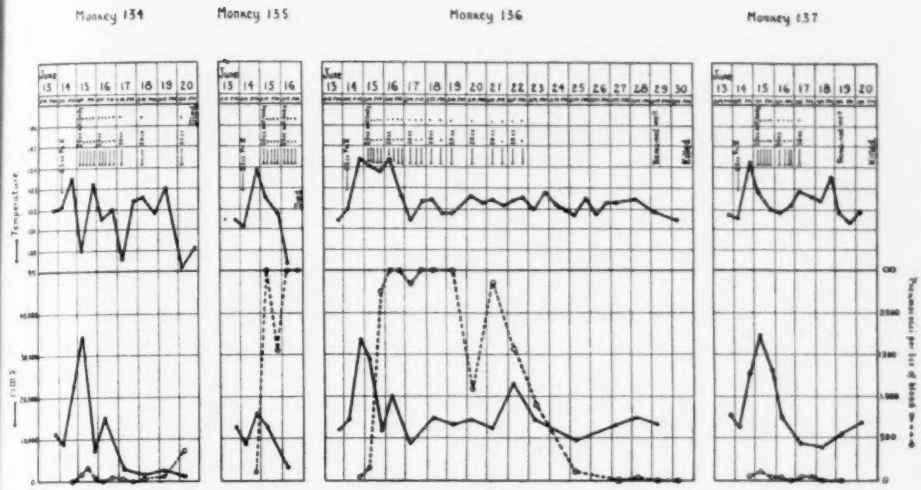


Chart 12.—Treatment of monkeys with pneumococcus type II pneumonia with Huntoon's antibody solution.

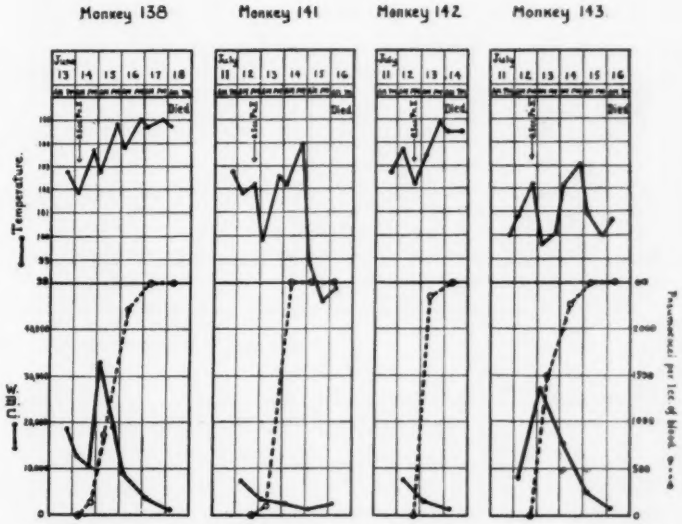


Chart 13.—Pneumococcus type II pneumonia in monkeys that were not given antibody solution.

both monkeys. The control monkey received no antibody and died on the fourth day with pneumonia and pneumococcus septicemia. From this experiment, it was clear that Huntoon's solution was just as efficacious in type I pneumonia as type I serum had been.

Our next experiment was concerned with the treatment of monkeys that had experimental type II pneumonia with Huntoon's solution (charts 12 and 13). Eight monkeys were subjected to experimental

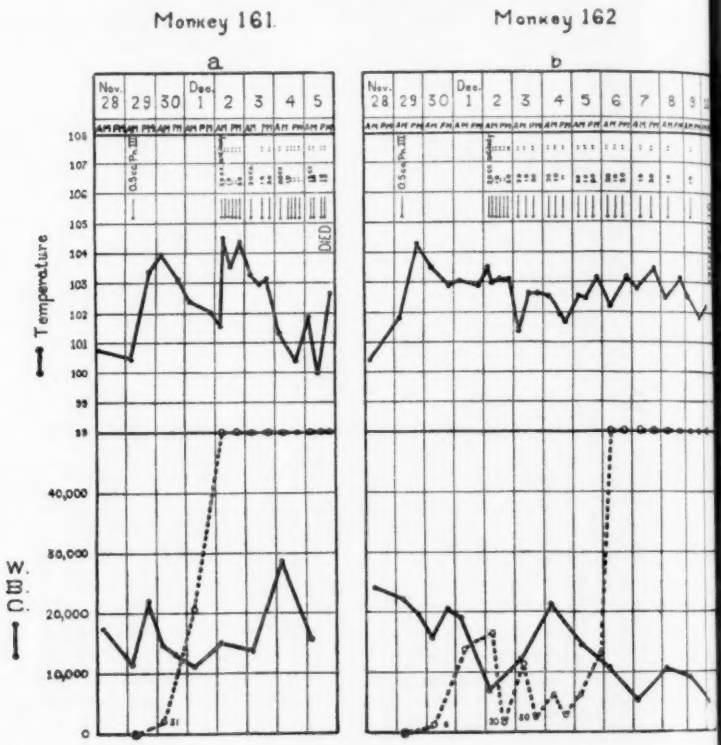
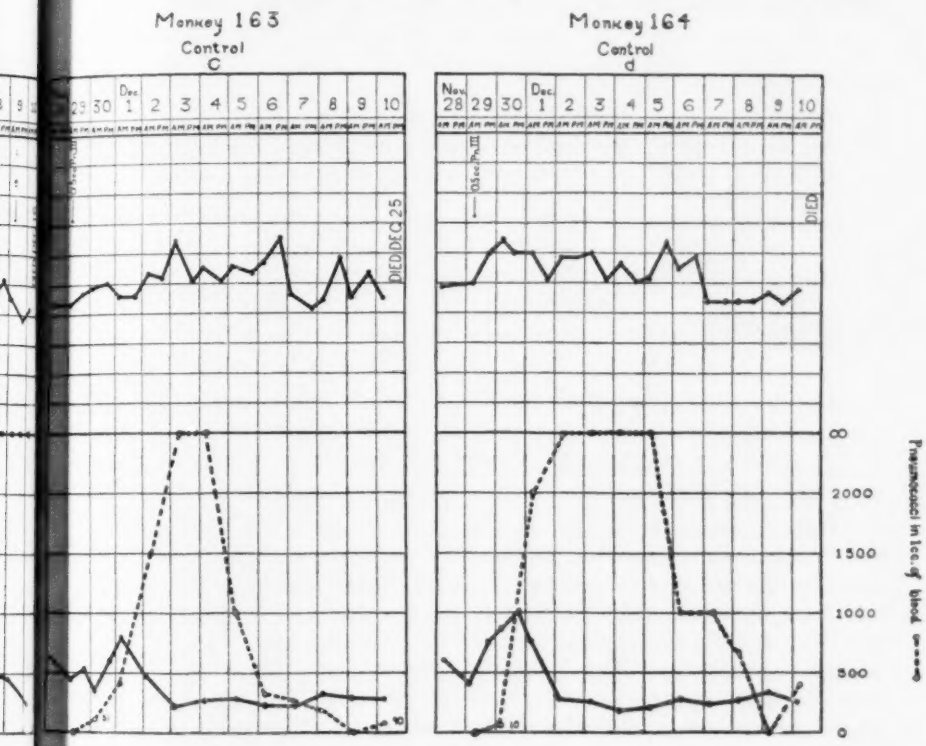


Chart 14.—Treatment of monkeys with pneumococcus

type II pneumonia by injecting a lethal dose of type II pneumococcus culture intratracheally. Twenty-four hours after injection the monkeys were sick. At this time, four of the monkeys (chart 12) were started on treatment with Huntoon's antibody solution, each monkey receiving 20 cc. intravenously four or five times a day. Larger doses were used than in the case of type I pneumonia for the reason that the antibody solution had less potency against type II than against type I pneumonia. As will be seen from the two charts, the four monkeys that did not

receive serum all died of pneumonia and septicemia from three to five days after the infection. Two of the monkeys that received serum recovered and two died. In the two that recovered, the antibody solution appeared to have the same effect that it had had in the type I infections, namely, it removed the pneumococci from the blood stream, but it should be noted that this was not done in so striking a manner as in the type I cases. In the other two monkeys treated septicemia persisted in spite of



II pneumonia with Huntoon's antibody solution.

treatment, and the monkeys died. The four controls (chart 13) died with pneumococcus type II pneumonia and septicemia.

As Huntoon's solution contained some protective substance against type III pneumonia, an experimental test was performed on monkeys with type III pneumonia (chart 14). Four monkeys (161, 162, 163 and 164) were given a lethal dose of type III culture intratracheally. All four promptly developed symptoms of pneumonia. Two of the monkeys, numbers 207 and 208 were started on Huntoon's solution about seventy-

two hours after injection. From three to five injections of from 10 to 20 cc. each were given daily thereafter. In spite of this treatment, both monkeys died with pneumonia and septicemia, one on the seventh day and the other on the twelfth day of their disease. The control monkeys (163 and 164) also died of pneumonia and septicemia.

From these experiments on monkeys, we were justified in concluding that Huntoon's polyvalent antibody solution was of great value in type I pneumonia, of some value in type II pneumonia and without value in type III pneumonia. As will be shown presently, these results tallied exactly with the results obtained in the treatment of patients with these types of pneumonia.

During the winters of 1920, 1921 and 1922, Huntoon's antibody solution was extensively tried out in the wards of Bellevue Hospital.[22] In six medical wards, every patient with lobar pneumonia who was

TABLE 3.—*Comparison of Death Rate in Series of Treated Patients and in Control Series*

	Antibody Wards			Control Wards		
Type of Pneumococcus	Cases	Deaths	Rate per Cent	Cases	Deaths	Rate per Cent
I	158	21	13.3	162	36	22.2
II	83	23	27.7	67	27	40.3
III	73	29	39.7	60	24	40.0
IV	110	18	16.4	121	29	24.0
Total pneumococcus	424	91	21.4	410	116	28.3
Streptococcus, etc.	48	24	50.0	35	12	34.3
Unclassified	36	14	38.8	47	20	42.5

admitted, regardless of type, was treated with antibody solution; in the other six wards, all cases of pneumonia were typed and the patients were carefully observed, but no antibody solution or other serum treatment was employed. Altogether, 424 patients with pneumococcus pneumonia were studied in the antibody wards and 410 in the control wards. Practically all of the patients in the antibody wards received antibody. The only exceptions were a few patients who died or had their crisis before antibody could be administered. The treated patients, however, have been included in the figures presented. Table 3 shows the results obtained. The most striking results, as in the monkeys, were observed in the type I infections. In 158 cases in which the patients were treated with antibody, the death rate was only 13.3 per cent, while in 162 control cases there was a death rate of 22.2 per cent. In type II infections, the figures were not so striking, but there was a difference. In eighty-three type II cases, in which the patients were treated with antibody solution, the death rate was 27.7 per cent as compared with 40.3 per cent in the

22. Cecil, R. L., and Larsen, N. P.: Clinical and Bacteriologic Study of One Thousand Cases of Lobar Pneumonia, J. A. M. A. **79**:343 (July 29) 1922.

control wards. Patients with type III infections were not benefited by antibody treatment, the death rate for the two groups being practically the same (39.7 vs. 40 per cent).

One of the most interesting and unexpected features of this therapeutic experiment was the effect of Huntoon's solution on patients with the so-called type IV pneumonia. One hundred and ten patients with pneumococcus type IV pneumonia who were treated with antibody, showed a death rate of 16.4 per cent; while 121 type IV controls had a mortality of 24 per cent. The probable explanation of this difference will be given presently.

When only cases in which the patients were treated early were counted, more striking figures were obtained. In table 4, the results

TABLE 4.—*Death Rates for Patients with Pneumococcus Pneumonia Receiving Antibody Solution Intravenously Within Forty-Eight Hours of Onset of the Disease. Control Shows Death Rate for Patients with Pneumococcus Pneumonia Admitted to Control Wards Within Forty-Eight Hours of Onset of the Disease*

Type of Pneumococcus	Cases Treated with Antibody			Control Cases		
	Cases	Deaths	Death Rate, per Cent	Cases	Deaths	Death Rate, per Cent
I	56	5	8.9	68	16	23.5
II	24	5	20.8	25	8	32.0
III	10	1	10.0	19	7	36.8
IV	24	4	16.6	45	11	24.4
Total	114	15	13.1	157	42	26.7

for patients that were treated with antibody solution within forty-eight hours of the onset are compared with those for the controls who were admitted to the hospital within forty-eight hours of the onset of the disease. In fifty-six patients with cases of early type I pneumonia who were treated with antibody, the death rate was only 8.9 per cent; sixty-eight controls admitted early had a mortality of 23.5 per cent. The death rate for twenty-four patients with type II pneumonia who were treated early was only 20.8 per cent as compared with 32 per cent in the controls. Even in the type III group the treatment appeared to have some effect, but the number of cases was too small to furnish reliable information.

The intravenous injection of Huntoon's antibody solution as originally prepared was practically always followed by a sharp thermal reaction from twenty to forty minutes after the injection. The patient began to shiver and soon had a severe chill. There was considerable cyanosis and dyspnea, and the patient often manifested extreme anxiety. The chill lasted from fifteen to thirty minutes. At its conclusion, the temperature showed a rise of 2 or 3 degrees, in rare instances even more. The high temperature usually persisted for only a short time (from thirty to sixty minutes). After that, there was a rapid fall, accompanied by profuse perspiration. These foreign protein reactions usually fol-

lowed each injection of antibody, but they tended to become less severe with each paroxysm. In three cases, the reaction following the injection of antibody appeared to be the immediate cause of death. In these three patients, the symptoms were severe: chill, followed by high fever; delirium; cyanosis; dyspnea; rapid, weak pulse; diaphoresis; congestion of the lungs, coma and death.

As one reviews this experiment in the light of further knowledge which has been gleaned during the five years since the experiment was carried out, it is obvious that we were obtaining something more than a purely specific effect. This is shown in the type IV group in which the

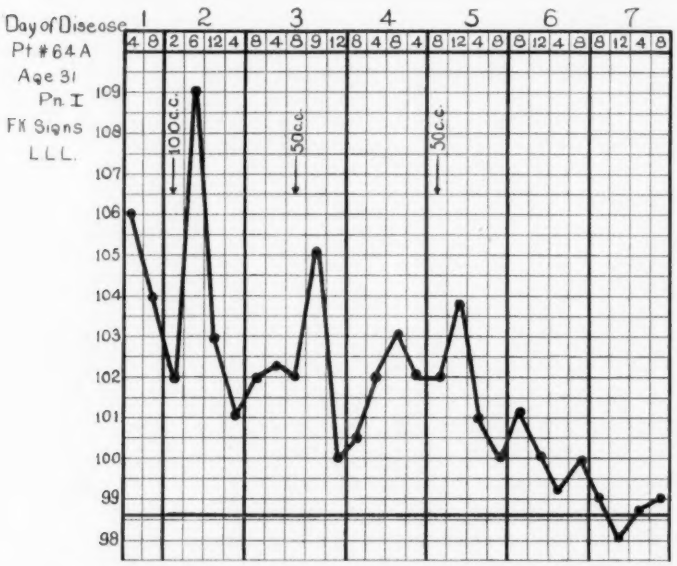


Chart 15.—Variations in temperature of a patient with pneumococcus type I pneumonia treated with Huntoon's antibody solution.

death rate was reduced to 16.4 per cent in treated patients as compared with 24 per cent in the untreated group. Undoubtedly, the specific antibodies contained in Huntoon's solution played an important part in the reduction of the death rate in types I and II pneumonia as the sudden and spectacular termination of symptoms after some of the thermal reactions which followed injections of antibody clearly indicates that the shock reaction itself was a therapeutic agent of considerable value in the treatment of these patients; in other words, the thermal reaction was a two-edged sword. It produced a brilliant therapeutic effect in some instances and was undoubtedly beneficial to the patient in most cases, but

the occasional fatalities which occurred after this mode of treatment rendered it too dangerous a weapon to be used. If there had been some method of controlling the serum, we could have continued to make use of its beneficent qualities, but there was no way of telling whether a patient would react with a mild or a severe chill. Chart 15 shows the variations in temperature of a patient with type I pneumonia and shows well the characteristic thermal reactions. Treatment was started on the second day of the disease. Three intravenous injections were given, and each was followed by a thermal reaction. Following the third reaction, the patient's temperature returned to normal and remained there.

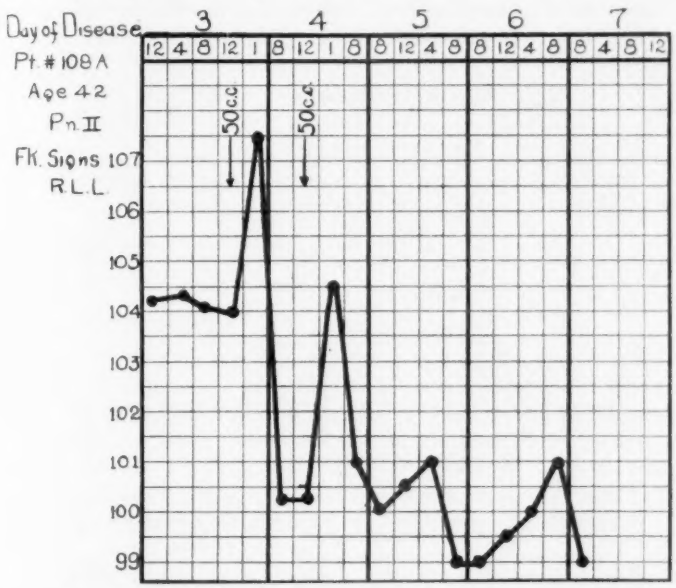


Chart 16.—Variations in temperature of a patient with pneumococcus type II pneumonia treated with Huntoon's antibody solution.

Chart 16 shows variations in the temperature of another patient treated with intravenous injections of antibody solution. This was a type II infection in which the first injection of antibody was given on the third day of the disease. Two injections of the solution sufficed to bring the temperature down to normal and to effect a rapid recovery. The reactions following the injections did not differ in any respect from those seen in the cases of type I pneumonia.

In some instances, Huntoon's solution appeared to have some clinical effect on patients with type III pneumonia, though the statistics for cases of type III pneumonia were not encouraging except when the injections were given early in the disease. Chart 17 shows the variations

in the degree of temperature of a patient with type IV pneumonia in which the intravenous injection of antibody produced a sharp reaction and apparently aborted the disease.

The next problem that presented itself was to obtain, if possible, the beneficial effect of Huntoon's solution without the disadvantage of the reactions. The simplest way to avoid the reactions was naturally to inject the solution subcutaneously instead of intravenously. Larger doses of antibody were employed for the subcutaneous treatment, from 100 to 200 being the initial dose, and in severe cases even 300 cc. was often given at one time. The average amount administered per case was 650 cc. This method of administering antibody was tried in the wards of Bellevue Hospital on alternate patients during the winters of 1922 and

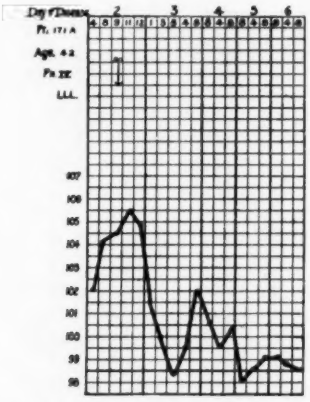


Chart 17.—Variations in temperature of a patient with pneumococcus type IV pneumonia treated with intravenous injections of Huntoon's antibody solution.

1923, and 1923 and 1924. The results were rather disappointing.[23] The subcutaneous method of treatment eliminated the chill, but appeared to have little effect on the course of the disease. The death rate for all types was almost as high for treated patients as for untreated controls. When only the early cases were compared, there appeared to be some difference. In table 5 the results for all patients with pneumococcus pneumonia who received antibody subcutaneously within forty-eight hours after the onset of the disease are compared with those for patients with pneumococcus pneumonia who were admitted to the control wards within forty-eight hours of the onset of the disease. There appears to have been beneficial effect, but the groups are small.

23. Cecil, R. L., and Baldwin, H. S.: J. Pharmacol. & Exper. Therap. **24**:1, 1924.

In the meantime, Dorothy Rhoades [24] had made a study of the fate of pneumococcus protective antibodies when injected into normal animals and man. She found that when pneumococcus antibodies are injected intravenously in a normal rabbit or man, protective bodies against pneumococcus type I are readily demonstrable in the circulating blood immediately after the injection and persist there for a variable period which may be two weeks or longer. On the other hand, she found that in animals injected subcutaneously with solutions of pneumococcus antibody, a certain amount of protective substance was usually demonstrable in the blood, but that in some cases antibodies do not make their appearance in the blood. The reason for this variation was not found.

TABLE 5.—*Death Rates for All Patients With Pneumococcus Pneumonia Receiving Antibody Solution Subcutaneously Within Forty-Eight Hours of Onset of the Disease. Control Shows Death Rate for Patients with Pneumococcus Pneumonia Admitted to Control Wards Within Forty Hours of Onset of the Disease, 1923 and 1923-1924 Series*

Type of Pneumococcus	Cases Treated with Antibody			Control Cases		
	Cases	Deaths	Death Rate, per Cent	Cases	Deaths	Death Rate, per Cent
I	23	4	17.3	23	6	26.0
II	13	5	38.4	27	15	55.5
III	7	4	57.1	21	10	47.5
IV	40	7	17.5	28	8	25.0
Total	83	20	24.0	99	39	39.2

Realizing the serious objection which the thermal reactions presented to the general use of solution of pneumococcus antibody, Huntoon had been trying to eliminate the chill-producing substance from his extract; after considerable experimentation, he succeeded in removing the greater part of it without decreasing in any way the potency of his product. The antibody solution now obtainable in the market can usually be administered intravenously without causing a chill, particularly if doses of 50 cc. are adhered to; large doses, from 100 to 150 cc. are still apt to produce a chill, and unfortunately, the large doses are often needed. Indeed, I doubt if the protective substance against types II and III pneumococcus in Huntoon's solution is sufficient to affect materially the course of a severe infection, that is, without causing a chill; if the dose is large enough to cause a chill, it may have a favorable effect not only on fixed types, but even on type IV infections. What the solution of antibody now needs is increased concentration. In a highly potent form, it would be an almost ideal product for the specific treatment of patients with the fixed types of pneumococcus pneumonia.

24. Rhoades, D.: Hyg. Tab. Bull., no. 141, 1925, p. 31.

FELTON'S CONCENTRATED ANTIPNEUMOCOCCUS SERUM

In 1924, Felton [25] published his first report on the isolation and concentration of the specific antibodies of antipneumococcus serum. In preliminary tests, Felton found that the protective substance was always associated with the water-insoluble fraction of serum, that is, the globulin. After various experiments, Felton [26] found that the largest return of immune bodies could be obtained from antipneumococcus serum by simply diluting one part of serum in ten parts of water. Taking advantage of this fact, he was able to isolate the specific antibodies and redissolve them in a concentrated form. The concentrate, of course, contained serum globulin in addition to the immune bodies. Actually these earlier lots of Felton's serum contained 3.5 mg. of nitrogen per cubic centimeter. The technic employed by Felton was as follows:

> One liter of serum is slowly poured into 15 liters of agitated, cooled distilled water, and the precipitate is allowed to settle over night in the icebox. The supernatant fluid is then syphoned off, and the flocculent precipitate is washed with the same volume of cooled, distilled water used for the precipitation. The suspension is again permitted to settle for twenty-four hours. Once more, the supernatant liquid is syphoned off and the white sediment collected by means of a Sharpless centrifuge. The compact white residue in the bowl of the centrifuge is taken out and dissolved in one-half molecular sodium chloride. If the solution is not clear, it is then passed through a Berkefeld candle. The resulting filtrate is a slightly opalescent fluid, free from sediment. By the use of this technic on a number of different antipneumococcus serums, concentrates two or three times stronger in immune bodies than the original were obtained. Even greater concentration, however, has been secured by using other solvents than sodium chloride, such as tartaric acid.

The first lots of Felton's serum were subject to the disadvantage which has already been described in connection with Huntoon's solution. Intravenous injections of even small amounts were nearly always followed by thermal reactions. Felton, however, like Huntoon, has discovered methods of eliminating the chill-producing substances. At the present time, not more than 10 per cent of patients have thermal reactions after intravenus injection, an incidence which is no higher than that following the intravenous injection of the original serum.

One of our first experiments with Felton serum was a test of its efficacy in the control of experimental pneumococcus pneumonia in monkeys. The variations in temperature and the number of white blood cells for four monkeys that were infected with lethal doses of pneumococcus type I pneumonia are shown in chart 18. Twenty-four hours after infection, three of the monkeys were started on intravenous injections of Felton's type I serum. The fourth monkey served as a control.

25. Felton, L. D.: Boston M. & S. J. **190**:819, 1924.
26. Felton, L. D.: J. Infect. Dis. **37**:1, 1925.

By referring to the charts, it will be seen that in the case of the three monkeys that received the serum, a striking effect was produced in the course of the disease. The temperature dropped and the pneumococci disappeared from the blood, just as in a previous experiment in which the monkeys had been treated with type I serum or with Huntoon's antibody solution. The three monkeys that were treated with Felton's serum made a complete recovery, while the control monkey developed a fulminating septicemia and died on the fourth day.

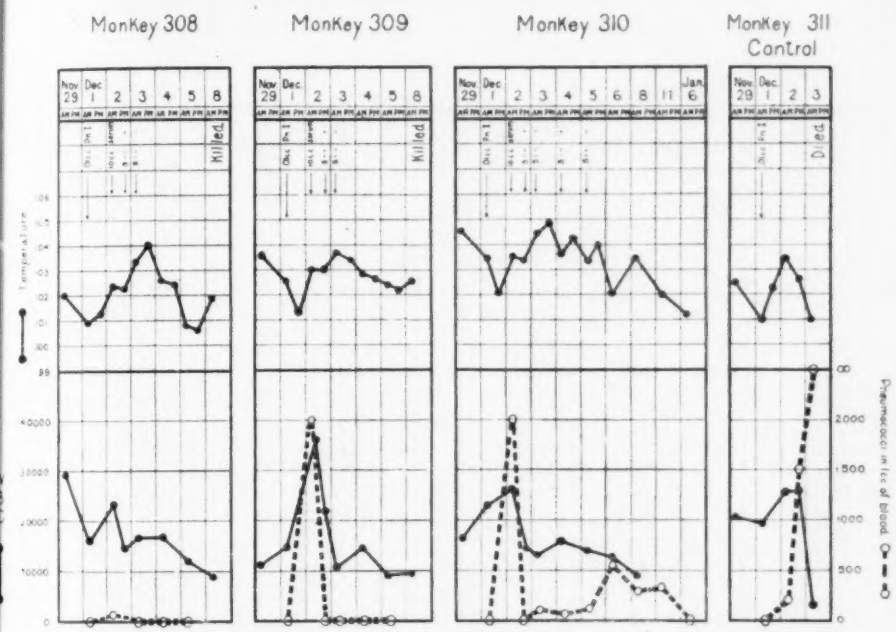


Chart 18.—Three monkeys (308, 309 and 310) with pneumococcus type I pneumonia were treated with Felton's type I serum and recovered. The control (311) did not receive the serum and died.

We then began to try out the Felton serum on patients in the wards. Dr. Felton supplied us with a polyvalent serum of high potency against pneumococcus type I, and a fairly high potency against pneumococcus type II. The polyvalent serum was administered intravenously as soon as the diagnosis of pneumonia was made. If bacteriologic examination revealed a pneumococcus type I or type II infection, we changed to monovalent serum, either type I or type II, as the case might be. If the laboratory reported pneumococcus type III or type IV, serum treatment was discontinued.

The clinical effect of Felton's serum on type I infections is often striking Chart 19 shows the variations in temperature and the number of pneumococci per cubic centimeter of blood of patients with type I pneumonia who were treated on the fourth day with two intravenous injections of Felton's type I serum. It will be noted that on the morning of the fourth day, the patient's blood was entirely free from immune bodies. Four hours after the first injection of serum, the blood showed a large amount of protective antibodies. The two injections of 10 cc. of serum were given in the evening. The patient's blood maintained a high content of immune bodies, and on the following morning the crisis occurred.

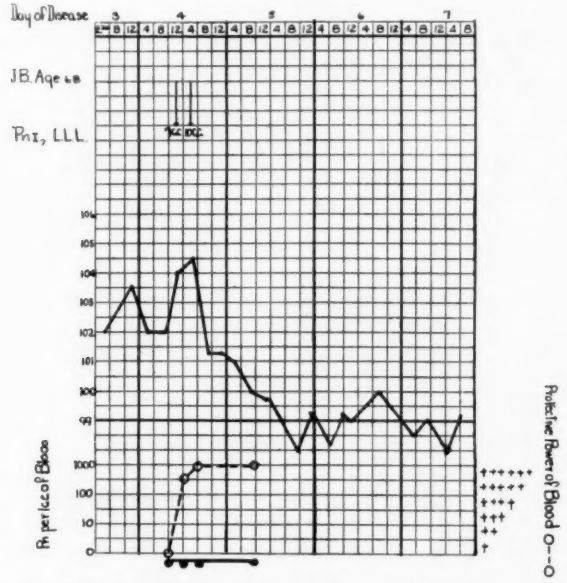


Chart 19.—Variations in temperature of a patient with pneumococcus type I pneumonia treated with Felton's concentrated type I serum.

In chart 20 the results are given for another patient with type I pneumonia admitted on the first day of his disease with a temperature of 102.5 F. and well marked septicemia (10 colonies to each cubic centimeter of blood). At this time the blood was entirely free from immune bodies. Six intravenous injections of Felton's serum were given on the first day. On the second day the blood was sterile and contained a large amount of protective antibodies. This state of affairs continued, and on the morning of the third day the patient had a crisis.

It is evident from these charts that small doses of Felton's concentrated serum are capable of achieving the same results that are obtained

by large doses of Cole's type I serum and Huntoon's antibody solution. The temperature drops, bacteria disappears from the blood and the crisis is hastened. Similar effects have been produced in patients with pneumococcus type II pneumonia, but we have found that in the case of type II infections, it is important that treatment should be started early.

Statistical evidence concerning the value of Felton's serum is not yet convincing, as the number of patients treated has not been sufficiently large. During the past winter in Bellevue Hospital, alternate patients with pneumonia have been treated with Felton's serum.

Patients who had type I and type II infections were treated intensively; those with type III and type IV received only the initial injections

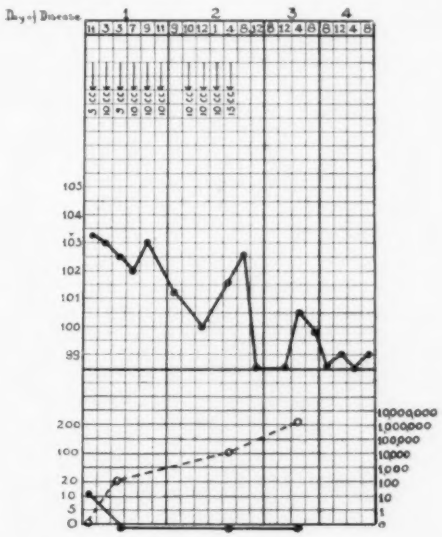


Chart 20.—Variations in temperature of a patient with pneumococcus type I pneumonia with septicemia treated with Felton's concentrated type I serum.

which were given before the types were determined. In table 6, patients that received serum are compared with those that did not receive any. In this table, patients dying within twenty-four hours after being admitted to the hospital have been excluded in both groups. In forty-eight cases of type I pneumonia in which the patients were treated, the death rate was 20.8 per cent; in the control group, the death rate was 29.5 per cent. In the type II group, treatment appeared not to have any effect, the death rate in the treated series being almost identical with that in the untreated series (48 per cent versus 46.1 per cent).

In table 7, patients that were treated during the first four days of their disease are compared with controls admitted to the hospital during the first four days of the disease. This table demonstrates clearly the importance of every treatment. The death rate in the treated patients with type I pneumonia is only 11.1 per cent, and the patients with type II pneumonia that received serum early show a mortality of 23.3 per cent as compared with 42.1 per cent in the control cases with type II pneumonia.

Incidentally, this table illustrates the perils of statistics. Nine patients with type III pneumonia in the untreated series show a mortality

TABLE 6.—*Death Rates of Patients with Pneumococcus Pneumonia Receiving Felton's Serum, Compared with Alternate Cases in Which Serum was not Given*

	Treated			Untreated		
Type	Cases	Died	Percentage of Mortality	Cases	Died	Percentage of Mortality
I	48	10	20.8	61	18	29.5
II	25	12	48.0	26	12	46.1
III	18	6	33.3	32	6	18.7
IV	66	16	24.2	43	14	32.5

TABLE 7.—*Death Rates of Patients Treated with Felton's Serum During the First Four Days of Disease, Compared with Controls Admitted During First Four Days of Disease*

	Treated			Untreated		
Type	Cases	Died	Percentage of Mortality	Cases	Died	Percentage of Mortality
I	18	2	11.1	21	4	19.0
II	11	3	27.3	19	8	42.1
III	4	2	50.0	9	0	0.0
IV	28	6	21.4	14	5	35.6

rate of zero! Ordinarily the death rate in this type of pneumonia is 40 per cent. For some reason the patients with type IV pneumonia who receive an injection of Felton's serum show a considerably lower death rate than the control cases. This figure, however, is only slightly below the usual death rate of patients with type IV pneumonia. The death rate in the control cases of type IV pneumonia is abnormally high in this particular series.

These figures indicate clearly that Felton's serum has a definite effect on type I pneumonia, this effect being most marked when the serum is administered early. The evidence in type II pneumonia is not so convincing, but judging from the effect in individual cases in which the serum administered early has sterilized the blood and caused a clinical

improvement in the patient, one would seem justified in concluding that Felton's serum will sometimes be of value in type II pneumonia, especially when administered early in the disease.

This conclusion is supported by the statistics which show a comparatively low death rate for patients treated with serum during the first four days of the disease. It must be admitted, however, that the number of cases is still comparatively small.

GENERAL SUMMARY

I shall now consider the comparative merits of these various specific agents. One thing I think is clear, and that is that the principle involved is the same in all. It has been proved by our investigations and those of others that death in pneumonia is accompanied in a large number of cases by pneumococcus septicemia, and vice versa, that septicemia is usually followed by death.

In this review, instances have been presented in which the sterilization of the blood in pneumococcus type II pneumonia in man followed and seemed to be due to the administration of some form of homologous antipneumococcus serum. I have also tried to show that the presence of protective substance in the blood is of distinctly favorable import in prognosis. Protocols have been presented which show that it is possible, through the administration of pneumococcus type I or type II immune bodies, to establish a balance of protective substance in the blood of the patient. I have referred to the work of Avery and Heidelberger who have shown that the pneumococcus produces a soluble substance which has the property of absorbing or neutralizing the productive antibodies produced by the patient.

It has also been pointed out that in the blood of pneumonic patients, either with or without pneumococcus septicemia, the specific soluble substance may be present and display the same faculties for neutralizing immune bodies. The recent studies of Robertson and Sia with leukocytes and immune serum indicate that the specific protective bodies are necessary for the phagocytosis of pneumococci.

The chief function, then, of antipneumococcus serum is bacteriotropic, that is, an opsonization of the bacteria, preliminary to their destruction by the leukocytes. If the blood stream is filled with "soluble substance," it is evident that the patient's own efforts to develop an adequate supply of immune bodies will often be neutralized by the soluble substance of the pneumococcus. If the immune serum is administered early and the administration continued for a sufficient length of time, the soluble substance may be neutralized and an actual balance of immune bodies established in the patient's blood. By such an achievement, the pneumococci are properly prepared for phagocytosis, and bacteremia is prevented.

The patient with pneumonia complicated by pneumococcus septicemia needs a great deal of help. The difficulty at present is that the patient often needs more help than one dares to give him with the agents at one's command. One hesitates to inject enormous quantities of horse serum into the veins of a sick man. The possibility of a chill or some unexpected antiphylactic reaction is enough to inspire the careful physician with a dread of intravenous serum therapy.

Of great importance, therefore, in the practical application of anti-pneumococcus serums are the problems of purification and concentration of the specific agent. Huntoon's pneumococcus antibody solution is almost entirely free from horse protein, but it lacks sufficient concentration. Felton's antibody extract has the concentration but lacks the entire freedom from horse protein which Huntoon's solution possesses. Both products sometimes produce a chill when large doses are injected intravenously.

Most of this discussion has dealt with the specific treatment of patients with type I and type II pneumonia, the two groups in which the outlook for a successful specific therapy is most promising. In the case of pneumococcus type III pneumonia, the serum and serum derivatives have thus far contained such small amounts of protective substance that their therapeutic value is practically nil. My co-workers and I have never been able to save monkeys with type III pneumonia, and we have been unable to sterilize the blood in the type III pneumonia of man by specific therapy. The death rate in patients with type III pneumonia who were treated with specific type III antibodies has been almost identical with that of the untreated patients. Specific therapy for type III pneumonia has little to offer at the present time.

In type IV pneumonia one is dealing, not with one group of pneumococci, but with a large number of biologically different types. In view of the type specificity of the protective substance, it is obviously impossible to make a type IV serum from a single strain of type IV pneumococcus. In Cecil and Larsen's series of patients treated with Huntoon's antibody solution, there was a decided lowering of the death rate in the treated patients with type IV pneumonia. Striking results in type IV pneumonia were also obtained by Conner [27] in his series treated at the New York Hospital with Huntoon's antibody solution. It is probable that the good results obtained in both of these statistics were due to the foreign protein or to (shock) reactions which occurred after the intravenous injections. The clinical results with Huntoon's antibody solution in type IV infections have not been striking since the chill-producing substances have been eliminated. It might be possible, of course, to prepare a polyvalent pneumococcus type IV serum by immunizing a horse against a number of pneumococcus type IV strains. This, however, is a problem for the future.

27. Conner, L. A.: Am. J. M. Sc. **164**:832, 1922.

SUMMARY

From the evidence submitted, it is clear that antipneumococcus serum and its derivatives, when administered under the proper conditions, are capable of exerting a definite influence on the course of pneumococcus type I and type II pneumonia. A specific therapy for these two types of pneumonia is, therefore, theoretically sound. The practical application of the specific treatment of pneumonia is still handicapped, however, by certain defects in the serum itself or in the derivatives from it. Whatever the serum or serum derivative used, the necessity of early and adequate treatment cannot be too strongly emphasized. In our experimental work on pneumococcus pneumonia, the value of large doses of serum has always been strongly impressed on us.

With numerous investigators now studying pneumococcus infections, there is every reason to believe that each succeeding year will shed further light on the problem. During the past decade much progress has been made, but much more still remains to be done before a thoroughly satisfactory specific treatment is achieved.

IMMUNITY TO TUBERCULOSIS*

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Every year about a hundred young women begin a three year course in the nurses' training school at Ullevaal in the Municipal Hospital of Oslo. While in training, some of these young women contract tuberculosis in its different forms. To ascertain whether or not this disease was more frequent among the pupil nurses than among people in other environments, my chief, Dr. Olaf Scheel, in 1924, began making Pirquet's tuberculin cutaneous tests on these nurses. Since then the investigation has continued, and this report is the result of this and other researches which have followed. The report will be divided into three parts in which the following facts will be considered: (1) the number of nurses with positive and the number with a negative reaction to the Pirquet test on entering the school; (2) the number and the classification of nurses contracting tuberculosis. (3) Can we prevent tuberculosis among nurses?

PIRQUET TEST

The reaction of the nurses to the Pirquet test on admisson to the hospital will be shown in the diagram. With a few exceptions, the nurses were between the ages of 20 to 25, and all were in good health.

Thus table 1 shows that on entrance to the school 52 per cent of the pupils gave a negative reaction to the Pirquet test and 48 per cent gave a positive reaction. The results of these tests are in sharp contrast to the prevailing opinion relative to the spread of tuberculosis among civilized people. It is generally believed that practically every one is infected with tuberculosis during childhood or youth, so that a negative reaction to the Pirquet test is seldom found after the age of 20.

Extensive investigations have been made among the children in the public schools of Oslo. These have shown that at the age of 9 about 85 per cent of the children gave a positive reaction to the Pirquet test. In the country districts, the number of positive reactions was less. The general opinion has been that one seldom finds a negative reaction among the inhabitants of the cities at the age of 18. Most of the material for the nurses training school comes from the cities, the pupils having resided in the city for a longer or shorter period of time prior to entrance to the school.

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* From Ullevaal, the Municipal Hospital of Oslo, Department of Internal Medicine; Dr. Olaf Scheel, chief physician.

* Preliminary report given before the Interstate Post Graduate Assembly of North America, Oslo, Norway, June 11, 1927.

In order to check the results of the investigation on pupil nurses, other groups of people of the same age were tested with the Pirquet reaction. Seventy-nine medical students were examined prior to their entrance into hospitals and 222 military recruits; 47 per cent of the medical students gave negative reaction to the Pirquet test and 53 per cent gave a positive reaction; 55 per cent of the military recruits gave a negative reaction and 45 per cent gave a positive reaction. These investigations confirmed our observations on pupil nurses in that about 50 per cent gave negative reactions to the Pirquet test. The old opinion relative to the frequency of the positive Pirquet test in adult life seems to be wrong.

The question may well be asked, Why is the percentage of negative reactions so great at the age of 20? One possibility is that special conditions in the children examined may have caused a higher percentage of positive reactions than among children in general; this percentage perhaps does not increase to more than 50 before the person reaches the age of 20. This is, contradicted however, by the agreement of all the earlier

TABLE 1.—*Reaction of Nurses to Pirquet Test*

	Number of Nurses	Negative	Positive
1924	109	51	58
1925	114	72	42
1926	114	62	52
1927 until June	83	35	48
Total	420	220	200

researches on the subject. Thus there are two outstanding facts: 1. In late childhood and youth there are nearly 100 per cent positive reactions. 2. The positive reactions fall to nearly 50 per cent at the age of 20. This must mean that the reaction to the Pirquet test has changed in about 50 per cent of the earlier positive cases.

In attempting to explain this change, the conditions required to produce a positive reaction must be stated. First, the organism must be infected with living tubercle bacilli; second, the organism must react to the infection, as every healthy organism does. But there are several states that produce anergy: acute infectious diseases, puberty, grave anemia, etc., in short, conditions producing asthenia. These conditions were absent in the nurses and other groups examined, as all those tested were healthy at the time of the investigation. There is, therefore, scarcely any reason to believe that 50 per cent of the persons gave a negative reaction because of the lack of necessary strength to react. On the contrary, everything seems to indicate that those tested were in the best condition to react, as will be shown later. Thus there is but one known possibility left, that is, the infection itself, the primo con-

dition for the reaction, is lacking. This seems to be reasonable, because tuberculous infection can rapidly or slowly kill or be killed by the organism. The latter fate has befallen the infection among the nurses who gave a negative reaction to the Pirquet test. They were infected in childhood and gave a positive reaction to the test, but the organism has little by little conquered the infection, and between the ages of 20 and 25 the infection has burned out; the positive reaction to the Pirquet test caused by the childhood infection has disappeared, and they are again virgin soil for a new infection, just as they were when they were new-born.

This is only a theory, but it agrees with the pathologic-anatomic researches. The late Dr. Jens Bugge of the Pathologic-Anatomic Institute of the University of Oslo has shown that there were no signs of living tubercle bacilli in about 30 per cent of the sections in which traces of tuberculosis were found.

The theory of a burned out infection and virginal soil for a new infection is mentioned in the second part of this paper.

TABLE 2.—*Number of Nurses with Tuberculosis That Gave Positive Reactions and Number That Gave Negative Reactions to the Pirquet Test*

	Positive	Negative
1924	0	13
1925	2	17
1926	1	14
1927	0	4
	3	48

NUMBER AND CLASSIFICATION OF TUBERCULOUS NURSES

The total number of cases of tuberculosis that have developed in the pupil nurses is fifty-one, but they have not been found in equal numbers among the groups that gave positive and negative Pirquet reactions. The distribution is shown in the table.

As far as the rest of the nurses who originally gave a negative reaction to the Pirquet test are concerned, only one of the 1924 class, two of the 1925 class and six of the 1926 class still gave negative reactions. I shall refer later to the group which started in January, 1927. But it can now be stated as certain that in the course of one or two years, the reactions of practically all of those who formerly gave a negative reaction to the Pirquet test have become positive, and that about 20 per cent of them have contracted a tuberculous infection. This seems to show that they were uninfected and presented a virgin soil when they began their hospital service, and that they soon contracted an infection.

If now the two groups are considered, the healthy nurses who gave a positive reaction as a result of an old infection, and the healthy nurses with a negative reaction who soon became infected, it is seen that fresh

tuberculous infection causes the greater number of cases of tuberculosis; an old infection seldom develops into the disease. In other words, the tuberculous infection generally shows its tendency at once either to conquer the organism or to be conquered by it and to establish immunity, which is expressed by the Pirquet reaction.

Should this matter be considered with special regard to the Pirquet reaction, it would seem that a positive reaction in a healthy person shows immunity against tuberculosis. It would then seem natural to attempt to obtain a positive reaction without infecting the subject with virulent tubercle bacilli.

IMMUNITY TO TUBERCULOSIS

I have succeeded in obtaining a positive reaction to the Pirquet test by using the avirulent tubercle bacilli (Bacille-Billie-Calmette-Guerin). This was originally a tubercle bacillus, typus bovinus, of general pathogenicity which Professor Calmette [1] and his collaborators at the Pasteur Institute of Paris brought to nonpathogenicity without loss of other special peculiarities, especially the tuberculin-producing capacity. They did this by cultivation on a medium of potato-glycerin plus 5 per cent of bile through 230 passages in thirteen years.

The nonpathogenicity of this bacillus has been tested in every way by inoculations of all kinds into various animals. It is interesting to note that inoculation of huge doses into thousands of guinea-pigs and anthropoid apes has not caused the development of tuberculosis in any case. Of still greater importance is the fact that the animals thus inoculated with nonvirulent bacilli have acquired resistance against infection by virulent tubercle bacilli and are immune as long as this infection lasts.

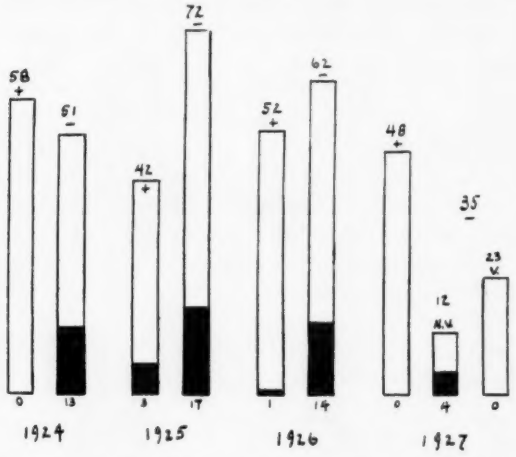
This is in agreement with Koch's theory of immunity to tuberculosis, namely, that as long as a person is infected with it, he cannot acquire a new infection.

In the fight against tuberculosis among cattle in France, this method has been used with much success. Professor Calmette has tried it on human beings and found it to be suitable for only those who were not already infected. Because of the widespread belief that every one has an infection earlier or later in childhood or youth, Professor Calmette selected for his tests young children most threatened with tuberculosis, namely, new-born children of mothers with open tuberculosis. In this group the mortality was extremely great. Statistics show that when a child remained with the mother the death rate during the first year was 125 per cent in France, excluding Paris; in other countries, it was as high as

1. Calmette, A., et al.: Presse méd. **33**:825 (June 20) 1925; **34**:241, 1409, 1926. Calmette, A.: Presse méd. **34**:897 (July 17) 1926.

72 per cent. With few exceptions these children are free from infection at birth. Professor Calmette gave each child 1 centigram of a suspension of bacilli by mouth three times at intervals of three days, the first dose being given as soon after birth as possible, before infection from the mother. To date, about 30,000 children have received this treatment in France and in her colonies, and it has not been reported that harm has resulted. Among the 1,200 children closely observed and controlled for a year or more, the mortality from tuberculosis dropped to 1 per cent.

Because the BCG bacilli are not harmful to children or animals, and because the BCG bacillus infection seems to induce resistance or immunity to tuberculosis in children, I began to use it on nurses who gave a negative reaction to the Pirquet test and who were supposed to be in the same



Pupil nurses grouped according to von Pirquet reaction showing incidence of tuberculosis. Numerals at the top of the columns indicate the number of nurses in each group; + indicates that the Pirquet test was positive; — that the Pirquet test was negative; *N.V.* in column eight shows number not inoculated; *V*, in column nine, number inoculated with BCG bacilli. Numerals at the bottoms columns indicate the number of cases of tuberculosis that developed in each group. Numerals, 1924, 1925, 1926 and 1927, indicate the year each group of nurses began training.

receptive state to tuberculous infection as new-born children. Because the oral inoculation of the new-born is based on the great permeability of the intestines, which soon disappears, subcutaneous inoculation was used. The dose given was much less because of the increased effectiveness and concentration of the bacilli when given subcutaneously, and about a year ago the first inoculation with 0.2 milligram of BCG bacilli in physiologic sodium chloride was used on two persons.

The first trial was made on a man, aged 33, (autotrial) to ascertain the effect of the inoculation if it were given by mistake to a person with a positive Pirquet reaction during a temporary negative phase due to some anergy-producing state. He gave a positive reaction to the test and was inoculated in the left arm. One hour later, there appeared "rubor, calor and dolor" about the site of inoculation and "functio laesa" of the arm. The symptoms continued for several days, then localized about the site of the inoculation, and after six days an abscess developed. The pus from it contained many tubercle bacilli. Attempts to treat the abscess by aspiration with the needle failed; and the surgical treatment was then begun and the abscess healed after nine weeks. Fever or symptoms of disease were not present during the entire period of the trial, which must be considered as a Koch's reaction, and showed that there was no risk in inoculating a person who gave a positive reaction to the Pirquet test.

The second trial was made on a boy, aged 4, who had repeatedly given negative reactions to the Pirquet test. The same dose and site were used for inoculation as in the foregoing case. Fever did not follow the injection, and the only untoward symptom was a little infiltration about the site of the inoculation, which suppurated two and a half months later; this suppuration continued for five months. At this point, the boy gave a positive reaction to the Pirquet test.

Because of the abscess formation in the cases reported, smaller doses, 0.03 mg., were given the nurses who gave a negative reaction to the Pirquet test. Some of these subjects developed a little infiltration, others did not have any reaction. Those who did not react were given another inoculation of 0.05 mg. a week later. Within four weeks after the second dose, there developed a small infiltration which was still present at the end of seven and one half months. All gave a positive reaction to the Pirquet test after six weeks. I consider 0.05 mg. the most suitable dosage, as this produced a local reaction, infiltration which signifies that the organism really has reacted to the inoculation. This method has been used in about forty cases.

The result of the inoculations has been, namely, all those who were inoculated gave a positive reaction to the Pirquet test after a sufficient length of time. The time between the inoculation and the appearance of the reaction has been from eleven days (a boy, aged 4, given a dose of 0.2 mg.) to eight weeks, averaging from about four to six weeks. About one half of those inoculated had an infiltration the size of a pea; of these, three suppurated; the suppuration was easily controlled. The others who were inoculated did not have a reaction, local or general, except that the Pirquet reaction became positive. The intensity of the reaction has varied from 1 or 2 sq. mm. along the scratch, to 10 mm. of rubor and infiltration. The meaning of this difference in reaction is not at this time apparent. The duration of the positive reaction to the Pirquet test and the allergy expressed by it is not known; in all cases that I have been able to follow it has been stabile through the period of observation up to one year (in one case).

One group on which the tests were made I shall mention especially, that is, the group of nurses with a negative Pirquet reaction who started their hospital training in January, 1927. There were twenty-three nurses. They were informed of the earlier results of the research and were asked whether they wished to be inoculated with the Calmette bacilli. Twelve wished to be inoculated, and this was done as soon as possible after they entered the service. Before inoculation and during the first weeks following it, they were not allowed in the tuberculous wards, so as to protect them as much as possible against malignant tuberculous infection during the period immediately following vaccination. Eleven of them refused to be inoculated. The result was that up to the present time, four of the eleven with negative reactions to the Pirquet test who refused vaccination and who worked in the hospital among its tuberculous patients have a tuberculous disease, but of the twelve who were vaccinated and whose reaction to the Pirquet test in this way became positive, none has a tuberculous disease.

SUMMARY

Researches among nurses have shown that at the age of 20, the age at which tuberculosis shows the greatest frequency, about one half of those examined give a negative Pirquet reaction; among these, with few exceptions, the cases of tuberculosis occur. When nurses who gave a negative reaction to the Pirquet test were inoculated with Calmette's avirulent tubercle bacilli, the Pirquet reaction became positive. The preliminary experience with those inoculated seems to show that inoculation coincidentally produces some resistance and immunity to virulent tubercle bacilli. Time and further observation will prove or disprove this theory.

AGRANULOCYTIC ANGINA

REPORT OF A CASE

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LOS ANGELES

Agranulocytic angina was first reported by Schultz [1] in 1922. In June, 1927, Kastlin [2] reported two cases and reviewed forty-one previously reported. Since Kastlin's monograph, Linthicum [3] has reported two cases and Finnegan [4] one. Of the forty-seven cases reported (including the one given below), forty-three (91 per cent) terminated fatally. Thirty-seven (78 per cent) occurred in females, the majority of whom were past the age of 40. In only one case was there a history of a previous attack.

The disease is characterized by an acute onset with a temperature of from 101 F to 105 F., with great prostration, sore throat or mouth and severe headache. It is sometimes accompanied by chills, general muscle pains, vomiting, herpes, bleeding from the oral and vaginal mucosa and jaundice. A membrane is usually present either in the region of the tonsils or along the gingival margins. In the fatal cases, the external genitalia are often involved, which may progress to deep ulceration. Usually glandular involvement is not marked. The blood picture shows a marked reduction of the number of granular leukocytes. *Bacillus pyocyaneus* has been isolated in a number of cases, and in none of these has recovery taken place. In several cases the bone marrow was found to be cell poor and the spleen enlarged at autopsy. However, these observations are not constant.

REPORT OF A CASE

History.—Mrs. K., aged 58, came under observation at 7 p. m., July 23, 1927. She complained of fever and sore throat. The family history was unimportant. The past history showed that there had always been a tendency to infections following any abrasions of the skin. For several years, she had had attacks of pain beginning in the right gluteal region and radiating down the back of the leg. On Feb. 1, 1927, she developed a sore throat. The pharynx, uvula and tonsils were covered with white patches. The uvula and tongue were swollen. A rash characterized by slightly raised red spots about the size of a dime covered the entire body. The rash did not become vesicular or pustular, but it caused great itching. This condition lasted for ten

1. Schultz, Werner: Deutsche med. Wchnschr. **48**:1495 (Nov. 3) 1922.
2. Kastlin, George J.: Agranulocytic Angina, Am. J. M. Sc. **173**:799 (June) 1927.
3. Linthicum, Fred H.: Pyocyanic Stomatatis with Agranulocytic Leukopenia, Calif. & West. Med. **27**:78 (June) 1927.
4. Finnegan, Frank P.: J. Missouri M. A. **24**:258 (June) 1927.

weeks. A temperature of 100 F. to 104 F. was present throughout this period. It receded gradually. During the entire attack, frontal and occipital headache was present constantly. The patient was told that she had a heart murmur at this time. The pain in her right gluteal region increased, with local swelling and tenderness. The swelling was incised, and a large amount of pus was obtained. During the following twenty-eight days, her general health was good, and the patient gained 20 pounds (9.0 Kg.); then suddenly she developed a similar rash, accompanied by anorexia and severe prostration. The next day the throat became sore. The entire pharynx was covered with a white membrane; the uvula was swollen and stiff; the "entire throat discharged pus." The temperature was 100 F. to 104 F. She had severe headache and nausea, and frequency of micturition with burning. At the end of two weeks, all of the symptoms had disappeared except the painful micturition.

The present attack began on July 22, 1927, with a similar rash followed by sore throat. Essential points in the examination when the patient was first seen (July 23) were as follows: a few small rose colored herpetic areas on the wrists about the size of a dime. The tonsils were slightly enlarged and red. There was some swelling behind the right tonsil; there was not any evidence of fluctuation; neither exudate nor a membrane was present. The cervical glands were slightly enlarged and tender. The temperature was 103.2 F., and the pulse rate 90. Results of examination of the heart and lungs were negative. On July 24 and 25, the condition was unchanged, except that the patient complained of more severe pain on swallowing. On July 26, a small amount of thick tenacious mucopus was removed from the region of the right tonsil. During the day, the temperature dropped from 104.4 F. to 101 F. There was great difficulty in swallowing.

Examination.—A complete physical and neurologic examination by Dr. E. L. Armstrong showed that the heart and lungs were normal. A slight enlargement of the spleen was noted, otherwise the results of the examination were negative. On July 27, all symptoms increased. The throat became filled with stringy mucopus, and a few small grayish patches on the pharynx appeared to be easily removable without bleeding. The uvula was slightly swollen. The genitalia were not involved. There was no pathologic process in the heart, and a few crepitant râles were heard in the lungs. The temperature was 102 F. to 104 F. On July 28, at 4 a. m., the heart became irregular and at 5 a. m., the patient became comatose; she died at 9 a. m.

Laboratory Examination.—On July 25 an examination of the urine showed a small amount of albumin, a trace of sugar, a great deal of diacetic acid, fine granular and a few epithelial casts and pus cells.

On July 26, the blood examination revealed: erythrocytes, 5,555,000; hemaglobin, 86 per cent; color index, 0.77; leukocytes, 660; lymphocytes, 82.5 per cent; polymorphonuclears, 16.0 per cent, and basophils, 1.5 per cent. The amount of nonprotein nitrogen was 42 mg. per hundred cubic centimeters of blood. The Wassermann test was negative. Culture and smears from the nose and throat showed abundant growth of mixed flora. Smears from the mouth were normal.

Tests for *Bacillus diphtheriae,* Vincent's bacillus and *B. pyocyaneus* were negative. Blood examination made at 9 a. m., July 27, revealed: icterus index, 16.0 per cent; leukocytes, 340; lymphocytes, 92 per cent; polymorphonuclears, 8 per cent. Tests at 5 p. m. showed: leukocytes, 280; lymphocytes, 94 per cent, and polymorphonuclears, 6 per cent. Changes had not occurred in the urine. Cultures from the nose and throat were negative for *Bacillus diphtheriae,* Vincent's bacillus and *B. pyocyaneus.* A blood culture was negative.

At 8 a. m., July 28, the leukocyte count was 310.

Treatment and Course.—Treatment apparently did not have any effect on the condition. The first three days the throat was treated daily with 25 per cent silver nitrate and 10 per cent mercurochrome-220 soluble every three hours. During the last three days, a mouth wash of 70 per cent alcohol was used every three hours. A blood transfusion of 500 cc. was given on July 27; a reaction did not occur.

Autopsy.—Autopsy did not aid in the diagnosis. A mild, cloudy swelling of the viscera was present. The bone marrow was normal. A pure culture of *B. coli-communis* was obtained from blood of the heart and from the cervical lymph nodes; the organism was probably an agonal invader. Cultures from the throat after death showed the same flora as before.

COMMENT

The unusual features of this case were: (1) the history of two previous attacks; (2) the extremely low white blood count (210 per hundred cubic centimeters of blood), and (3) the fact that all viscera and the bone marrow were essentially normal.

The number of cases recently reported make it probable that this condition has been overlooked in the past and is more common than would seem from the paucity of the reports. The diagnosis can be made certain only by the blood examination. A marked leukopenia with a relative increase of lymphocytes is always present. Other symptoms are variable. The only case in which treatment has been of any value is the one reported by Finnegan. He believes that the blood transfusion cured his patient. A 70 per cent alcohol gargle has been recommended on the theory that it is germicidal for *Bacillus pyocyaneus.*

This report is given with the hope that this malady may be recognized more frequently and that eventually a satisfactory treatment may be discovered.

1920 Wilshire Boulevard.

STUDIES IN ASTHMA

II. AN ANALYSIS OF TWO HUNDRED AND THIRTEEN CASES IN WHICH THE PATIENTS WERE RELIEVED FOR MORE THAN TWO YEARS *

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As I have recently suggested,[1] the causes of asthma are manifold and group themselves like the parts of a tree. The main trunk of the tree represents a fundamental basis which may be called the "asthmatic state" and explains why some persons have asthma and others do not. The roots of this tree represent the causes of the asthmatic state, but so far only one root—that of inheritance—can be recognized. Upward, the tree divides into two main branches—the extrinsic and the intrinsic. Both of these can be traced through numerous subdivisions. The extrinsic branch divides into pollen, animal danders, domestic and other dusts, foods and other substances, all of which are readily recognized. Twigs on these smaller branches represent, of course, the particular pollens, the different animals, the different dusts, etc. The main intrinsic branch is likewise subdivided into groups of causes, chiefly "bacterial" and "reflex," as outlined in recent papers.[2]

Such a conception as this tree represents merely a working basis, and the theory suggests a number of questions for consideration. When one speaks of "ragweed asthma" or "cat asthma," does one imply merely that the person has inherited or acquired a biologic hypersensitiveness to ragweed or to cats, and that the asthma which results from contact with ragweed or cats is a manifestation of this hypersensitiveness and that this alone can explain the entire situation? On the other hand, should it not be recognized that asthma is a characteristic symptom-complex, and that in spite of its manifold causes, its essential mechanism is the same in each case? Is it not reasonable perhaps to assume that the various substances which precipitate the attack in the patient are merely exciting causes and represent only the trigger mechanism which fires the attack, but do not tell how or why the gun was loaded?

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* From the Medical Services and Anaphylaxis Clinic of the Massachusetts General Hospital.

* The expenses of this investigation were met by an anonymous donation known as the "M. G. H. Asthma Fund."

1. Rackemann, F. M.: Asthma, in Cecil, R. L., and Kennedy, F.: Textbook of Medicine, Philadelphia, W. B. Saunders Company, 1927, p. 481.

2. Rackemann, F. M.: Studies in Asthma: 1. A Clinical Survey of 1,074 Patients with Asthma Followed for Two Years, J. Lab. & Clin. Med. **12**:1185, 1927.

In other words, can asthma be regarded merely as an expression of a changed immunologic reaction to various foreign substances and should one not include the conception of a changed physiologic process which makes the new immunologic reaction possible? How else can one explain the fact that a small number of persons become hypersensitive to ragweed or to cats, when such an immense number of other people are exposed, apparently, to an equal extent, but never have trouble?

Many authors appear to assume that all asthma is of extrinsic origin and that if the exact cause cannot be identified, the difficulty lies in their own faulty technic. I maintain, however, that this point of view is unnecessary, and I hope to show that in certain cases extrinsic causes can be excluded, and that the exciting cause (the trigger mechanism), as well as the fundamental cause, lies within the patient's body.

To throw light on these various questions, a clinical study of asthma was undertaken on a pretentious scale. The gross results of this study have been published recently,[2] with a classification of the patients and a statement of total end-results obtained by a follow-up of 1,074 patients with asthma who were seen at the Massachusetts General Hospital and in private practice prior to Jan. 1, 1925, and who, therefore, have been followed for two years or more.

These results include the reports from 213 patients who maintain that they have been entirely free from asthma and without treatment for at least two years, and who are therefore designated as "cured," a word which is used for convenience, but always with skepticism.

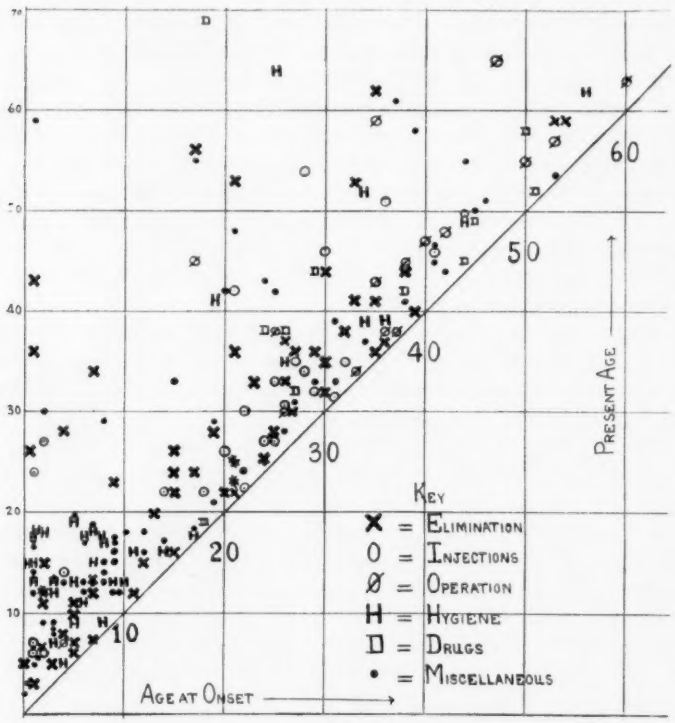
A further analysis of these 213 "cured" cases ought to throw light on the method of "cure" and consequently on the mechanism of asthma. It should show whether "cure" was accomplished simply by removal of the "trigger" mechanism, which was therefore only temporary; or whether it depended on a fundamental change in the physiologic and immunologic reactions. Further, it should suggest the general nature of the "asthmatic state" so far as the method of "cure" would rule in or out such possibilities as an anatomic nerve reflex between the nose and bronchi, some endocrine imbalance or perhaps an obscure chemical change.

Incidentally, it was anticipated that this particular study would demonstrate that the conception of a foreign protein excitant is not always necessary, and that it is proper to conceive that asthma has an exciting cause within the body; this cause is called "intrinsic."

With these thoughts in mind, the various groups of "cured" patients have been studied with several objects in view. First, to recall the classification and the clinical picture of the patients at the time of their first examination, over two years ago; second, to study the method of "cure"; third, to analyze critically the extent of "cure" and fourth, to note the incidence of foci of infection and other lesions in the "cured" cases, partly to rule out these lesions as factors in the mechanism of the asthma

in the particular case and partly to determine to what extent "cure" can obtain in the face of these lesions.

The accompanying chart shows the age at the time of "cure" of these patients, plotted against the age at onset. The apparent method of "cure" is shown by the different symbols indicating elimination, operation and other factors, as will be discussed. "Cures" fall into all age groups, but are particularly numerous among those who began to have asthma during childhood.



Method of cure in two hundred and thirteen "cured" patients.

The percentage relationship of the cases in which the patients were "cured" to the total number of cases is shown in table 1, which gives the figures in each decade of onset. According to these figures, the change of "cure" apparently is not much greater when asthma begins in one decade than when it begins in another, except for the better prognosis among those with an onset during the first decade. Sixty-five per cent of all children with asthma were boys, and boys formed 73 per cent of the "cured" children; so that boys have a greater chance of developing

asthma and also have a greater chance of recovery. In other terms, 30 per cent of 201 boys were "cured," while only 20 per cent of 112 girls were "cured." In the later decades, up to the sixth, the number of men and women was about the same, both in total numbers and in "cured" cases.

These figures fail to show any regular relationship between "cure" and age or between "cure" and sex, and suggest that since the occurrence of "cure" in this series is so evenly distributed, it is merely incidental.

The relation of "cure" to classification is more important. The methods of classification have been described in the first study,[1] and in that paper it was shown that 425, or 40 per cent of the 1,074 patients had extrinsic asthma, while 499 or 46 per cent had intrinsic asthma; the remainder being unclassified. The "cured" patients formed 25 per cent of the extrinsic group, 18 per cent of the intrinsic group and 10 per cent of the unclassified group.

TABLE 1.—*Percentage Relationship of the "Cured" Cases to Total Cases*

Decade of onset	0-9	10-19	20-29	30-39	40-49	50-59	60+	Total
"Cured" patients	84	29	45	32	14	8	1	213
Males "cured"	61	13	19	13	7	2	0	115
Percentage	73%	45%	42%	41%	50%	25%	0%	54%
Total patients	313	166	202	183	129	59	22	1,074
Total males	201	75	82	87	73	36	14	568
Percentage	65%	45%	41%	48%	57%	61%	64%	53%
Percentage of "cured" to total patients	27%	17%	22%	17%	11%	14%	5%	20%

EXTRINSIC ASTHMA

In the extrinsic group, the "cured" cases occurred among patients with various subclassifications of the disease, as shown in table 2.

Simple (Uninfected) Pollen Asthma.—In this group of seventy cases, there were thirteen patients who were "cured." These thirteen cases included ten adults, varying from 22 to 57 years of age, and three children. All had asthma which was limited to the pollen season and which was due to ragweed in seven cases, to grasses in three and to both ragweed and grasses in three others. The "cure" of patients with asthma was brought about in eight cases by treatment with ragweed which was combined with treatment with grass pollen in the three cases in which the patients were sensitive to grass. Every patient was treated during several successive seasons, usually four or five; but two patients, aged 43 and 23, were treated for only two years; however, they have not had asthma or hay-fever since the last treatment, which was given five and eight years ago, respectively. Apparently, the result is permanent. Four others report a complete relief from asthma, but do not mention hay-fever. Two of the eight patients treated, however, still have some

symptoms of hay-fever and give positive skin reactions to ragweed, so that they are not "cured."

In addition to the eight patients "cured" by injections, there were five "cured" by other means. One patient attributes his "cure" to a tonsillectomy, although two years previously he had had a series of injections of grass pollen with good results that year, while in four instances a definite cause for "cure" was not apparent. Two of these four patients had previously had several series of injections of pollen extract followed by some improvement. One of these patients, a man aged 22, who had had asthma all of his life, gave positive reactions to

TABLE 2.—*Classification of 213 "Cured" Cases Showing the Method of "Cure"*

	Method of "Cure"								
Clinical Classification	Elimination	Injections	Drugs	Operations	Hygiene	Miscellaneous	Total "Cured"	Grand Total	Percentage "Cured"
Pollen asthma	0	8	0	1	0	4	13	70	19
Infected pollen asthma	0	2	0	0	1	2	5	39	13
Summer asthma, negative tests	1	0	0	0	2	7	10	32	31
Animal asthma	16	3	0	0	1	2	22	60	37
Infected animal asthma	0	0	0	0	0	0	0	13	0
Mixed and unidentified	16	1	0	0	0	5	22	121	18
Mixed and unidentified with negative tests	16	0	0	0	1	1	18	37	49
Extrinsic specials	9	3	0	0	2	3	17	53	32
Total extrinsic cases	58	17	0	1	7	24	107	425	25
Bacterial asthma	1	6	5	2	3	8	25	202	12
Bacterial asthma in children	1	2	0	0	15	16	34	90	38
Reflex asthma, not nose and throat	0	1	0	4	7	6	18	66	27
Reflex asthma, nose and throat only	0	0	0	9	0	1	10	40	25
Cardiac asthma	0	0	1	0	0	0	1	56	2
Bronchitis and emphysema	0	0	1	0	1	1	3	45	7
Total intrinsic cases	2	9	7	15	26	32	91	499	18
Unclassified asthma	2	0	4	0	0	9	15	150	10
Grand total	62	26	11	16	33	65	213	1,074	20

skin tests with ragweed injected intradermally. He was treated with ragweed extract and was greatly improved. After two years of supervision, he was lost track of, and on returning after an interim of five years without treatment, he gave negative reactions to pollens by scratch test. At this time, he was having attacks of asthma infrequently, and never severely, as before treatment. Although he was not treated for asthma, two years later this condition disappeared and he has not had a return of symptoms during the three years since then.

Lesions in the nose and throat were found in three of the thirteen patients, the tonsils being a focus of infection in two and the antrum in the third. One of the patients has been mentioned as being "cured" by operation, and the other two have become symptom-free in spite of the fact that they had not been treated for this condition, which suggests

that the focus as found was acute and self-limited instead of chronic and persistent; in any case, it had nothing to do with the symptoms.

Infected Pollen Asthma.—The fact that in this group of thirty-nine patients only five were "cured" illustrates the obstacle which secondary infection puts in the way of successful treatment. The five patients were all adults "cured" at or before the age of 40. Three were sensitive to ragweed, the fourth to grasses and the fifth to both. Three of the patients were treated with pollen extracts; two were "cured" by this alone, while the third was not completely relieved until her bad teeth were extracted. Of the two patients not treated with pollen extracts, one, a woman, aged 34, still has hay-fever, but she has not had asthma since resection of her nasal septum, three years ago. The other, a boy now 19 years of age, "outgrew" his trouble coincident with marked improvement in his general health at the age of 17, two years ago. He has lived in the same house all his life.

Summer Asthma with Negative Tests.—Thirty-two patients were included in this group, ten of whom were "cured," ten were improved, ten remained in the same condition and two died. The diagnosis, unconfirmed by any tests, was based solely on the history of asthma limited to the summer months. In the whole group, only 10 per cent of the patients had a positive family history in contrast to the figure of 47 per cent for all extrinsic cases; curiously, this 10 per cent included only one of the ten "cured" patients.

Five of these ten "cured" patients were healthy adults, and five were children with asthma of only a few years' duration and limited entirely to the summer months.

The results of the physical examination were normal for all except one child who had a barrel-shaped chest; a roentgenogram showed increased shadows of the hilum. Three patients, one adult and two children, had a focus of infection in the tonsils; one was operated on with temporary relief and one without improvement.

The method of "cure" varied. Two of the children had their tonsils removed and also received hydriodic acid, which was given to two others together with a vaccine. Three adults also received iodides, with vaccine in one instance. These seven cases are described as miscellaneous because the method of "cure" was so uncertain. One of the children did not receive any treatment other than general directions for rest and a more regular dietary, and this "cure" by such improved hygiene was duplicated by one of the adults who likewise was not given any other treatment. The "cure" in one of the adults is credited to "elimination," because although the patient received iodides, one cannot be sure that the change in address was not important; perhaps her case should be listed in the unidentified group, in spite of the seasonal character of her attacks.

The duration of "cure" in the ten patients has been from two to five years; in only two cases has it followed a change in environment, which might imply an escape from some offending pollen not included in the tests.

Animal Asthma.—Animal asthma of the simple type offers the best prognosis of any important subgroup. Of a total of sixty, twenty-two patients were "cured" and thirty-two were improved. In the group of twenty-two "cured" patients, sensitiveness was manifested toward cats in eleven cases, toward horses in six, toward dogs in two, toward rabbits and guinea-pigs in one and toward a combination of two or more animals in the remaining two instances.

Specific treatment with the corresponding animal dander was attempted in seven adults, but the "cure" was attributed to the treatment in only two cases of horse asthma and in the one case of rabbit and guinea-pig asthma. The history of the latter patient has been published in detail.[3] Toward the end of the treatment, each of these three patients could tolerate exposure to the particular animal without developing symptoms, and the three have been free from asthma for ten, four and two years, respectively. In sixteen other cases, the animal itself was eliminated, usually after treatment had failed, and this elimination was obviously the effective method of "cure." Three additional patients have become free from asthma without reference to the specific skin tests found. One of these was a school boy, aged 11, thin and sickly, who gave good reactions to test with cat hair, horse dander and feathers. When taken out of boarding school and left at home, he improved remarkably and has not had asthma during the past four years; he has gone back to school. His sensitiveness to animals was undoubtedly the exciting cause of the asthma, as shown by his prompt improvement at home; but his low vitality and chronic fatigue were perhaps of fundamental importance. Two men sensitive to horse hair do not know how they became symptom-free. One, who was formerly extremely sensitive, has not exposed himself to horses and now is free from asthma. The other is a hostler who is still at work and without symptoms. The last man gave a history of asthma during late August and September and frequently during the winter, but he did not react to skin tests with ragweed. The diagnosis was made by the slight test to horse dander, in consideration of his occupation.

A focus of infection was found in the nose and throat in only one of the twenty-two asthmatic persons who were sensitive to animals. This patient had received several doses of cat hair extract and also had had her tonsils removed. Relief from asthma did not occur, however, until some time after this treatment, when the cat was eliminated. For the past two years she has not had any trouble.

3. Rackemann, F. M.: Asthma, M. Clin. North America **7**:765, 1923.

Repetition of skin tests since the "cure" was established has been accomplished in only three of these patients, and in all of them the reaction was still positive, so that obviously the fact of "cure," in a literal sense, is open to grave question.

While positive reactions to skin tests with animal dusts usually indicate a hypersensitiveness which is clinically important and thus lead to the relief from asthma by the elimination of the particular animal, these tests are occasionally misleading so far as the corresponding animal is of no clinical importance; whatever the cause of disease, the patient can be successfully treated without regard to the hypersensitiveness suggested. As will be discussed later, such tests may indicate past history rather than present illness.

When infection occurred with animal asthma, not one of the thirteen patients in this series was "cured."

Extrinsic Mixed and Unidentified Cases with Positive Skin Reactions.—There is another considerable subgroup of extrinsic asthma. The twenty-two "cured" patients in this group represent 18 per cent of the total 121 patients with mixed and unidentified types of asthma who gave positive skin reactions.

All of the patients reacted to several different extracts, most of which were extracts of different house dusts or house dusts in combination with other substances. Seven patients had hay-fever as well as asthma. Two gave a history of eczema during infancy, and one had hives after eating pickles or nuts or after drinking wine. Only seven had a positive family history.

Of these twenty-two patients, sixteen were "cured" by a change of environment. Although ten of them had been treated by the extracts indicated, with good temporary results, an absolute freedom from symptoms was not acquired by any of them until, by change in residence, the patient escaped from some unidentified dust which was the exciting cause of the condition. These patients were all young adults who were "cured" at or before the age of 40, with the exception of one child, a boy, aged 12, who gave positive skin reactions to tests with house dusts and feathers, and who had symptoms only when visiting certain farm houses during his summer vacations. He was "cured" by following the advice to keep away from these old farm houses. Five years later, when he returned to the clinic in answer to a follow-up letter, he was tested again with house dusts, feathers, animal dusts and other substances; the results were negative.

There were six other patients in this unidentified group whose method of "cure" is hard to define. One gave positive skin reactions to house dusts and to animals and was treated with these extracts with good results. She also had several bad teeth and some clouding of one antrum. Soon after the extraction of her teeth the antrum cleared up, and she became free from asthma. The second patient, a young man,

who gave strongly positive reactions to tests with mattress hair and house dusts, had round shoulders, a prominent abdomen, a flat chest full of wheezy râles and a traumatic obstruction of his nose. He was advised to discard his hair mattress, to take regular exercise and regular rest, to have his nose straightened and to take benzyl benzoate. By one, or all of these methods of treatment, he has become free from asthma, although he still lives in the same house and in the same environment. In the third case, a child, aged 9 years, whose asthma began at the age of 2, and who gave positive skin reactions to tests with food and house dusts, the "cure" is attributed to an acute pulmonary infection, because he has been free from asthma for eight years following an attack of whooping cough which occurred several months after his first visit to the clinic. The fourth patient, a man, aged 42, gave positive skin reactions to tests with epidermal dusts and also house dusts; he also had a history of coryza after contact with dogs. Strange to say, his skin reactions, although positive to tests with cats and feathers, were negative to those with dog hair. This man was obese, and his chest was filled with mucous râles. He was advised to avoid animals and was given eight injections of a stock hemolytic streptococcus vaccine. About a year later, he returned with a cold, but he did not have asthma at that time nor during the two years intervening since then. One of the two remaining patients (both adults) was given injections of the pollen to which he reacted, without apparent benefit. He did not return for further treatment, but about a year later the asthma ceased for no apparent reason, and he has remained symptom-free for the intervening four years. The last patient also gave positive skin reactions to tests with house dusts, animals and feathers. He became free from symptoms, although this was not effected by a change in residence or by any particular avoidance of animals or feathers; he was given a series of stock vaccines, with good results, which may have helped in a general way.

A focus of infection in the sinuses was found in seven of the twenty-two patients, and two were operated on with improvement. A focus in the tonsils was found in three patients, but operation was advised in only one. There were two patients with abscessed teeth, and both were improved following extraction of the teeth.

In this group, then, change in environment resulted in complete relief from asthma in sixteen of the twenty-two cases. Treatment with house dust extracts or with other substances to which the patients reacted when skin tests were made, brought about "cure" in only one case. While the patients in three of the remaining five cases were treated, this treatment cannot be credited with the "cure" which could in each case be better explained in another way.

Extrinsic Mixed and Unidentified Cases with Negative Skin Reactions.—An additional subgroup of thirty-seven patients includes eighteen

"cured" patients—a high proportion. (The diagnosis in the other nineteen was made because the asthma also bore a definite relation to environment. Most of the patients are now improved, but a few others still have asthma because they have returned to the old surroundings.) These patients present the same clinical picture as those in the preceding group, except that their skin tests, by the intradermal method and often by the scratch method as well, were negative or so slightly positive that they were disregarded.

Only three patients had manifestations of hypersensitiveness other than asthma; two had hay-fever and one, urticaria. There was a positive family history in only six instances.

Of the eighteen "cured" patients, fourteen have been relieved of their asthma since they changed their residence, in spite of the fact that none of them gave the slightest reaction to skin tests with dusts. One boy, aged 15, was "cured" when the carpet in his room was changed, "on general principles"; in spite of the negative reaction to skin tests, this boy was evidently hypersensitive to some foreign substance associated with that carpet. A baker, who associated his asthma definitely with the use of flour, but who gave entirely negative reactions to tests with wheat and other cereals, was improved by doses of extract and finally entirely relieved by changing his work so as to avoid wheat as a dust. In the remaining two cases, one patient was a girl, aged 13, whose asthma occurred only when she went to the family's summer home at the seashore. Advice in regard to cleaning and airing the house thoroughly, in addition to instructions for general hygiene (she was a thin, frail child) were followed by a "cure." In the other case, that of a child, aged 5, the "cure" cannot be definitely attributed to a change of environment, because this change followed soon after a series of injections of autogenous vaccine, which was already helping the patient. The extrinsic factor cannot be excluded, but neither can it be given full credit.

A focus of infection in the nose and throat was found in six of the eighteen patients. The sinuses were dull in four patients, and the tonsils were infected in three. Although operations were advised in several cases, they were never performed, except that in one case the patient had a nasal cauterization, which helped somewhat but did not effect the "cure." Abscessed teeth were not found.

Extrinsic Specials.—The term applies to an interesting group of fifty-three patients known to be hypersensitive to some definite substance contained in the food or in the environmental dust. Industrial cases belong in this group. In spite of known etiology, only seventeen of these patients have been "cured." In eleven of them the cause of asthma was food; in six others, it was a dust.

The eleven cases in which the patients were sensitive to food include eight children, four of whom were poisoned by eggs, two by fish, one by wheat as bread and, finally, an infant, who was so sensitive to cow's milk that a few drops would cause swelling of the mouth, vomiting and urticaria, and yet whose reactions to skin tests, in spite of this high degree of sensitiveness, have never been large. One of the patients sensitive to egg was a boy, aged 16, who had been extremely sensitive to eggs and nuts since infancy. His case is of interest because he presented a picture of hypopituitarism, and because he has been immensely improved by the feeding of an extract of anterior pituitary. He has not had asthma since egg was eliminated from his food. In the group of patients sensitive to food are also three adults; two are women, aged 40 and 38, who became hypersensitive to foods at the ages of 19 and 33, respectively. Both have remained free from asthma, one for three and the other for seven years, since the offending foods (in one case celery, nuts and fish; in the other case, wheat and oats) were eliminated from their diet. The third was a student, aged 22, who since infancy had had asthma caused by eggs, and who was so sensitive that it was necessary for him to watch his diet with greatest care. He was "cured" by a series of twenty-seven hypodermic injections of increasing quantities of egg-white in watery solution.

The degree of "cure" in these food cases is not perfect. Although the student just referred to has not had any treatment for two years and now eats an egg for breakfast every day and is no longer troubled by cake and puddings, his test to egg-white is still positive. The two women, however, do not dare to change their diet, although one regards herself as "one of the wonders of the world" and the other has "forgotten I ever had asthma." One of the eight children still gives positive reactions to skin tests with eggs, the reaction to the scratch test being as large as it was seven years ago; nevertheless, the child eats eggs without difficulty. Two other children who were sensitive to eggs are eating eggs without trouble, but the two who were sensitive to fish still hesitate to indulge in this food, although they are free from asthma. The child who was sensitive to milk is now drinking 24 ounces of cow's milk each day. The boy who was sensitive to wheat has recovered from his asthma after avoiding bread for a year and a half; the boy with pituitary dysfunction also eats every kind of food and is well.

From this it appears that positive reactions to skin tests can occur without any relation to the symptoms or lack of symptoms. On the other hand, the positive tests in these cases agree closely with the symptoms observed during infancy and childhood; consequently, it is obvious that they serve merely as a record of past experience and are without bearing on the present condition. This is entirely in accord with the

observations of Mackenzie [4] on bacterial hypersensitiveness as well as with the well known dependence of a positive tuberculin reaction found in adult life on a primary infection in childhood.

The six cases in which the patients were sensitive to dust include two children and two adults who have not had asthma since they discarded their feather pillows. The fifth case is that of a grocer who was sensitive to hops and malt and who has been "cured" by injections of malt extract. He is still at work in the same place. Finally, a housewife, aged 32, has not had asthma since a series of treatments with orris power extract, to which she gave good skin reactions.

Comment on Extrinsic Asthma.—The results as outlined bring out clearly that in most of these cases "cure" has been accomplished by removal or treatment of the trigger mechanism which fired the attack. Evidence concerning the permanency of the results is conflicting. While certain patients can return to the old environment or the old diet after a lapse of time and remain free from asthma, an equal or greater number remain hypersensitive, and symptoms return with repeated exposure.

In this last group, it is obvious that the "cure" is merely temporary and that, although the trigger may have been disconnected for a time, the gun remains loaded. In the other group, however, there is evidence not only that the trigger mechanism has been removed, but that the load in the gun has been modified.

What relation do these cases bear to the young patients who have "outgrown" their asthma? Does the material suggest any factors which may be common to all with a permanent "cure"? This subject will be discussed at the end of the paper.

INTRINSIC ASTHMA

Intrinsic asthma is more difficult to analyze. Indeed, the existence of such a type has been ignored by those authors who imply that every case of asthma depends on a hypersensitiveness of the patient to some foreign substance which may be unidentified either because the variety of test substances is not sufficiently extensive or because the technic is not sufficiently perfected. Such an attitude is not without reason.

The variety of test substances has been increased by the attention called to house dust by Cooke [5] and to various fungi and molds by Van Leeuwen,[6] and physicians in every large clinic are constantly identi-

4. MacKenzie, G. M., and Hangar, H. M., Jr.: A Study of Hypersensitiveness to Derivatives of Hemolytic and Non-Hemolytic Streptococcus, Proc. Soc. Exper. Biol. & Med. **21**:542, 1924.

5. Cooke, Robert, A.: New Etiologic Factors in Bronchial Asthma, J. Immunol. **7**:147, 1922.

6. Van Leeuwen; Storm; Bien, Z., and Varekamp, H.: Neuere Erfahrungen über Diagnose und Therapie von Ueberempfindlichkeitskrankheiten (allergische Krankheiten), Ztschr. f. Immunitätsforsch. u. exper. Therap. **37**:77, 1923.

fying various dusts met with in industries as materials to which the persons exposed will develop hypersensitiveness and symptoms. The large number of pollens which Duke [7] and Rowe [8] have identified as causes of hay-fever is being extended by many other workers. This improved technic has made it possible to show that certain patients formerly classed as having intrinsic or an unidentified type of asthma have extrinsic asthma and are hypersensitive to a previously unrecognized cause.

In addition to this improved technic, there are other reasons for considering an extrinsic cause in many persons who give negative reactions to skin tests. Chief among these is the fact of relief from symptoms when the patient moves from one environment to another, although reactions to skin tests with a wide variety of substances are entirely negative. This in turn suggests that a patient may have a clinical hypersensitiveness, confirmed by relief from symptoms when the cause is withdrawn, without giving positive reactions to skin tests. Such a conception would be of the utmost importance, but so far it cannot be proved. On the other hand, I believe, as outlined in a previous article,[2] that the clinical analysis of a certain number of patients leads to the logical conclusion that the cause of asthma in them must be intrinsic rather than extrinsic.

In the following discussion of the cases in the intrinsic group, a few patients with positive reactions to skin tests have been included, but, as will be seen, a study of the history and environment has failed to show any relation between the symptoms and the particular substance with which the test was made. In this connection, it has already been pointed out in discussing food asthma that positive reactions to skin tests may represent past history rather than present illness.

The division of patients with intrinsic asthma into subgroups is difficult. It is easy to conceive of asthma dependent on infections of the larger bronchi and to designate these patients as having "bacterial asthma." It is easy to distingiush a second large group in which the cause is in the body but outside of the bronchopulmonary tree and to assume that asthma is a reflex effect of some stimulus arising from this extrabronchial source. Both these conceptions serve simply as working hypotheses and are without a basis of experimental proof. In spite of this, the classification is useful because typical cases in the bacterial and in the reflex groups can be recognized clinically, and it is important to study them further in order to determine whether a total disregard of extrinsic factors is justified by the clinical evidence at hand. When, however, a

7. Duke, W. W.: Asthma, Hay-Fever, Urticaria and Allied Manifestations of Allergy, St. Louis, C. V. Mosby Company, 1925.

8. Rowe, A. H.: Pollen Counts in the East Bay Region with Studies on the Morphology of Pollen. Read before the American Society for the Study of Asthma, at Washington, D. C., May 16, 1927, to be published.

patient appears to have a bacterial asthma and at the same time is found to improve after the removal of a focus of infection, his classification becomes difficult and unsatisfactory.

The group of bacterial asthma is the most important and characteristic of intrinsic asthma. For convenience, the fifty-nine "cured" patients have been separated into twenty-five adults and thirty-four children (table 2).

The typical patient has had asthma since an early age. This asthma usually began after an acute infection of the respiratory tract and has occurred in well defined attacks since the onset, each lasting from one to four weeks in the fall, winter and spring, but rarely in summer. These attacks occur without change in environment or occupation and often begin with "a cold." They occur at first only in the fall and spring, but later become more frequent, so that the interval between may be shortened to one or two weeks. Nevertheless, the interval exists, and in it the patient is free from cough and asthma and is essentially well in the same environment while leading his regular life. The interval is important, particularly when the conditions are the same, because it argues against any constant extrinsic factor. It is true that patients with typical extrinsic asthma have intervals between their attacks, but analysis will show that these experiences are always directly associated with the particular exciting cause. Reactions to skin tests in the group, as a rule, are negative to the common dust substances by the intradermal method.

Among the twenty-five "cured" adults the onset of asthma was, for the most part, between the ages of 20 and 40, though one patient, aged 26, developed it at the age of 8 and six others during their teens, five of whom were not 20 at the time of examination.

A story of an acute infection of the respiratory tract immediately preceding the original attack of asthma was obtained in only ten patients. Two other cases are of particular interest and suggest the possibility that chronic as well as recurrent infection may lead to asthma. One patient, a girl aged 20, had had several attacks of pneumonia during childhood and had had a running ear since the last attack twelve years ago. Asthma began at the age of 19, in November, after she had had a persistent cold; during the preceding winter she had had a cough. When examined at the age of 20, she was thin and pale. Coarse musical râles were found at the bases of the lung, but the apexes were clear. She recovered from her asthma at the age of 21, following tonsillectomy; five years later, at the time this article was written, she stated that she has one or two bad colds each winter, but that she has not had asthma.

The second patient, a girl, aged 26, weighing 97 pounds (44 Kg.), had had osteomyelitis of the femur since the age of 9. She had her first attack of asthma at the age of 24, following a cold in October. The

second attack occurred the next year, in November, and the third the following October, just before she was examined in the clinic. The culture of sputum showed a hemolytic streptococcus in predominance, and a vaccine of this organism was given that winter (1923). She has not had asthma since then.

The extent of "cure" is shown by the fact that in eleven cases five years have elapsed since the last attack of asthma; in five cases, four years; in five cases, three years, and in three cases, two years, and only five of the twenty-four patients mention the presence of colds in their follow-up letters.

The reactions to skin tests were positive in four patients, but were not of any clinical importance.

The method of "cure" is almost impossible to identify because these were all difficult cases, and many methods of treatment were applied. Vaccines were used in eight of the twenty-five patients and were apparently responsible for the "cure" in six. Drugs are given the credit in five patients. (Potassium iodide in two, benzyl benzoate in one, emetine hydrochloride, subcutaneously, in one and a patent medicine in another.) Elimination has brought freedom from symptoms in one, and a change in residence has completed the "cure" started by potassium iodide. The remaining "cures" in thirteen patients cannot be explained. For example: A man, aged 44, who had had asthma in typical attacks since he had had influenza in 1918, and who had a barrel-shaped chest and thick yellow sputum, "has never been bothered with asthma" since gastroenterostomy for ulcer at the age of 48, three years ago.

A woman, aged 34, whose asthma began with a cold in May, a few months previous to her first examination, came to the hospital only once and did not receive treatment; however, she has not had asthma since a severe attack of influenza at the age of 36, nine years ago.

A thin, nervous business man, aged 48, who had had two attacks of asthma each year since the age of 21, was tested in a routine manner by the intradermal method and has not had asthma since that time, seven years ago. Incidentally, the reactions to these tests were entirely negative, and his bad teeth have not been extracted.

A Jewish attorney, aged 39, who had had attacks of asthma following colds since the age of 34, has been entirely well since the correction of his constipation and the institution of daily exercise. His "cure" is ascribed to this improved hygiene.

In addition to the twenty-five adults, there were thirty-four "cured" children with "bacterial asthma," twenty-five boys and nine girls. The age of onset was under 5 years in eighteen, and between 5 and 10 in sixteen. This onset occurred immediately after colds or bronchitis in sixteen instances, in seven of which the preceding illness was sudden and severe (pneumonia in three, whooping cough in one and a bad cold

in three others). Another patient dates the onset from tonsillectomy at the age of 3 years.

The physical condition of these children at the time of examination varied. Such notes as "undernourished," "pale and thin" and "poorly developed" appear in the records of fifteen, but a barrel-shaped chest with emphysematous lungs was noted only once—in a boy, aged 12, who had had attacks of asthma which occurred every few weeks throughout the year since the age of 7.

Diseased tonsils had already been removed in fifteen children, and three others showed infected tonsils at the time of examination. A tonsillectomy was performed in one case.

Evidence of sinusitis, with dulness of the antrums on transillumination or on examination by the roentgen ray, was found in five patients, all in their early teens, but drainage was not advised or attempted; the apparent method of "cure" was improving of the hygiene, although one boy, aged 13, was given a vaccine made from the staphylococcus found in his nose. This boy is now a mile runner on a university track team.

Eczema was mentioned in the past or present history of five of these children, one of whom gave a good reaction to tests with several cereals, including buckwheat, wheat and oats. Two other children had had urticaria, one of them after eating strawberries.

Skin tests, by the scratch method, using the common food and dust substances, were made on all but four of these "cured" children. In addition to the child sensitive to cereals, three others gave positive reactions to skin tests; one to wheat, one to feathers and one to animals—the last child, however, was never exposed to animals, and the method of his "cure" cannot be defined, although he has been under the constant supervision of an excellent practitioner.

The family history of thirteen children was positive, including four children with other allergy.

The permanency of "cure" in these children can only be surmised. Suffice it to say that seven have been free from asthma for five years or more, seven for four years, six for three years and fourteen for two years. It is interesting that of the seven children free for five years, the method of "cure" in four consisted solely of measures to improve the general condition and hygiene. None of these children had had any treatment by injections of vaccine or other substances, and reactions to skin tests were negative in all except in the case of the child with eczema who was sensitive to cereals, previously mentioned. Curiously enough, this particular group of seven children includes five of the other seven children with either eczema or urticaria.

In the entire group of thirty-four children, fifteen were "cured" by "general hygiene" without other measures and particularly without atten-

tion to foreign proteins. None of the fifteen had changed his address before the "cure" took place. Ten of the fifteen were undernourished, weak children, and the other five had bad habits of diet, rest and exercise.

In one case, the mother writes that she has discovered that asthma was due to eggs and apples, and that since her son has omitted them from his diet, he has not had any further trouble. This relation to certain foods was noted before the examination, and tests with active extracts were made on two different occasions, but the results were always negative. Incidentally, the boy was given four doses of a stock vaccine and had his tonsils removed, after which he was much improved. His "cure," however, did not begin until about four years after the time of observation. The relation to eggs and apples was evidently not extremely close, but the data in table 2 show the "cure" by elimination.

Two children were "cured" by vaccine, stock and autogenous, respectively. While it appears that this treatment was important, a comparison with other cases, particularly in the hygiene group, clearly shows the fallacy of claiming too much for any method.

Finally, there are sixteen children whose "cure" cannot be attributed to any one particular method. For example, a child, aged 10, whose father was asthmatic, had had eczema as an infant and attacks of bronchitis at the age of 4. In spite of tonsillectomy at the age of 6, attacks of bronchitis recurred, but asthma did not develop until he was 9, when the attacks were rather continuous from December to June. Reactions to skin tests, by both scratch and intradermal methods, were negative. He did not receive any treatment. The father writes that the attacks diminished in frequency and severity until they ceased at the age of 12. The boy is now 17 years of age.

Comment on Bacteria Asthma.—The analysis of the cases of these patients with bacterial asthma who have been "cured" leads to a number of observations.

Bacterial asthma as a diagnosis is adequate, since the theory of recurrent infections of the respiratory tract as a cause of asthma fits the clinical picture better than any other conception which can be considered at present. The clinical history presented by most of these patients is sufficient to rule out the causative factors dependent on hypersensitiveness to a foreign protein.

The method of "cure" does not suggest any underlying mechanism which is common to a majority of these patients, but it emphasizes two important points: 1. General advice based on a common sense effort to improve the general bodily condition—referred to here as "hygiene"—is of great importance, especially in children, and makes many of these patients amenable to treatment by any physician and without special equipment. 2. The tendency of children to outgrow their susceptibility to infections of the respiratory tract is definite.

Reflex Asthma.—The term reflex asthma indicates that clinically the cause of the asthma is based on a focus of infection in the body or on the faulty function of one or another organ. Table 2 indicates that in a total of 106, twenty-eight patients were "cured." These twenty-eight have been divided into those whose asthma was definitely attributed to a focus of infection in the nose and throat and those whose asthma was not attributed to this cause. The twenty-eight cases include eight children and twenty adults, both age groups including some patients with pathologic changes in the nose and throat.

Eighteen patients were "cured" of asthma which was designated as reflex from some source other than from the nose and throat. Six of these were children, all of whom were in poor general condition. In four of them asthma had been constantly present for from one to three years before examination, while in the two others, it had occurred in fairly definite attacks which were worse during the winter. The last two are, of course, analogous to the cases of bacterial asthma in children already mentioned, but they were originally placed in this reflex group because the immediate results of improving the general bodily hygiene, with directions for regulation of diet, rest and exercise were so striking. "Hygiene" accounts for the "cure" in all six patients, although three are listed as miscellaneous "cures" because one child had had vaccine treatment; another dates his "cure" from an attack of bronchopneumonia seven years ago, as since then his general condition has improved to a remarkable degree; and a third child, aged 11, not relieved by tonsillectomy at the time of examination, has not had asthma since an appendectomy five years ago, two years after his first visit to the clinic. Possibly, too, his slightly positive skin test to potato may have been important.

Only two of these children had a positive family history.

A focus of infection was found in three children—two tonsils and one sinus—but as appropriate treatment did not help them, these foci were disregarded.

The twelve adults varied in age from 16 to 62, and asthma was not developed before the age of 25, except in the two younger patients. The histories of these patients were atypical, as none of them told of clear-cut attacks characteristic of bacterial asthma. Reactions to skin tests were negative in all; two had changed their address, without causing any change in symptoms.

"Hygiene" was the method of "cure" in four of these adults who were advised to drink less tea, to eat more lunch, to lie down at noontime and to eat foods containing less carbohydrates and fats, respectively.

Operative treatment accounts for four more; a man, aged 45, and another, aged 55, have not had asthma since the removal of bad teeth; a woman, now 45, has been free from asthma for seven years, since her

gallstones were removed when she was 38 years old; and finally, a man, aged 62, who had a cholecystectomy, has not had asthma since the operation was performed two years ago.

In four patients, the method of "cure" is unidentified, chiefly because various methods of treatment, including a change in residence, were employed in each case. In them the designation of "reflex" calls attention to other conditions present. One had a positive Wassermann reaction, and, as antisyphilitic treatment seemed at first to prevent asthma, syphilis was considered a reflex cause. A careful comparison between the changes in the Wassermann reaction resulting from treatment and the changes in asthma did not show any constant relation between the two, and at the present time, although the patient is free from asthma, the Wassermann reaction is still strongly positive. At various times she has also been given vaccines, and she has had her teeth extracted. Another woman developed asthma during her first pregnancy, and had it again during the second, but she has not had asthma during the six following years. A laborer, aged 40, did not have any evident cause for asthma except bad teeth. He is now "cured," perhaps because his teeth have been removed.

Finally, a woman, aged 38, who had repeated attacks of asthma, each definitely connected with infections and relieved temporarily and in succession by appendectomy, by influenza during pregnancy and by removal of teeth and tonsils, has not had asthma since the administration of an autogenous vaccine three years ago.

The ten cases with a reflex cause in the nose and throat include those of two children. A girl, aged 12, who had asthma since an attack of influenza at the age of 9, had numerous polypi, dulness of the sinuses and badly infected tonsils. The polypi and tonsils were removed shortly after examination, with considerable improvement (six years ago); but her "cure" dates from "about three years ago," and as she gives a new address the well marked lesions were perhaps unimportant, and the original diagnosis of reflex asthma should more properly have been extrinsic asthma. Another child was, however, definitely "cured" by tonsillectomy, and two adults have remained free from asthma for four and five years since tonsillectomy.

The other six adults had nasal polypi and degrees of sinusitis, and in every one of these cases the "cure" dated from operative treatment seven, five, four, three, three, and two years ago, respectively.

Reaction to skin tests by the patients in these reflex cases were always negative.

Cardiac Asthma and Chronic Bronchitis with Emphysema.—The heading indicates that the asthma is associated with changes in the circulation or in the lungs which are organic, well recognized and permanent.

The 101 patients were elderly people, with the exception of seven young adults (now all in the forties) each of whom had a characteristic barrel-shaped chest, with clubbed fingers and chronic cyanosis.

While one of the patients with heart disease and three of the patients with emphysema have been relieved of their asthma, the word "cure" cannot be used even in the loose sense in which it has been employed in this paper. A woman, aged 65, with severe asthma since the age of 17, which was not affected by residence in California, Maine or Carolina, was given potassium iodide when first seen in 1921; since then she has had but a single attack of asthma (in 1925). She has, however, recently developed symptoms of decompensation and of angina pectoris.

Two of the patients with emphysema were women aged 53 and 61; both had hypertension and both have been free from asthma for two and five years, respectively, since taking potassium iodide. The other patient, a Jewish tailor, aged 42, had a history of wheezing for twenty years; he had a slightly barrel-shaped chest. After coming to the hospital he became comfortable, and he has not had asthma during the intervening three years. His symptoms were obviously exaggerated as was the original estimate of his emphysema.

UNCLASSIFIED ASTHMA

Unclassified asthma is self-explanatory. Only 10 per cent of the 150 patients were "cured"; these fifteen include thirteen adults and two children. Most of them were "cured" in some unidentified or miscellaneous manner. Drugs, however, were clearly responsible in four cases; iodide in two, acetylesalicylic acid in one and arsphenamine in an interesting case, in which asthma was due to a gumma of the larynx. Two patients were "nervous." One an apprehensive French maid, who had asthma, both in the city and in the country, has not had asthma since going home to France, three years ago, although she returned to the same family and to the same house in the United States one year later. The other girl was a temperamental Italian who went to Italy four years ago, and who has been well since then. Her case is classed as miscellaneous and not extrinsic, because her asthma was of only five weeks' duration and cleared up to a considerable degree after treatment with iodide and vaccines; this was two years before her departure for Italy.

The cases of nine other patients remain unclassified, chiefly because the method of "cure" was bizarre, consisting of the use of electricity or an unknown "patent medicine" or simply of examination and skin tests without any definite treatment; yet all of the patients had a definite history of asthma. A few other cases are so complex that any satisfactory analysis of the histories and manifold methods of treatment is hardly worth while.

COMMENT

In the foregoing discussion of "cures" among individual patients and groups of patients, it is evident that the study as a whole is hardly susceptible of accurate analysis. On the other hand, the study of certain patients is based on objective observations which are accurate. In some of them, the method of relief can be identified, and although its mechanism is unknown, the word "cure" can be applied to the end-results with some confidence.

Table 2 not only represents the methods of "cure" in the various subgroups of cases, but also shows the relative importance of all these various methods in all cases. Thus in the extrinsic group, the elimination of some external factor is the important method, although in a few cases, treatment of the patient with an extract of the corresponding substance has been effective. "Cures" by hygiene and by miscellaneous methods are particularly numerous in the intrinsic group in which specific methods have yielded disappointing results, but it is impossible to define, with any accuracy, the mechanism by which this "cure" was brought about.

In reviewing the work it is important to determine the number of cases in which the method of "cure" is known and in which a true cure can be claimed. Such a study applied to this particular series is handicapped by the fact that only in a few cases of the intrinsic group as a whole were the skin tests repeated after the "cure" was established. The fact, however, that in these few the reactions to the retests were always positive makes the thought of time cure in the group doubtful.

At the same time, attention is called again to the children with asthma caused by sensitiveness to eggs who later were able to eat eggs in spite of the fact that their reaction to skin tests with egg-white is still positive. This suggests the thought that other positive reactions to skin tests relate to past history rather than to the present illness. Such an idea must modify the definition of a "cure" and justify the belief in a true cure even in the presence of persistently positive skin reactions.

Many of the patients have been "cured" by a change to a new environment, which has allowed them to escape from the cause of the asthma; in these cases also it is difficult to tell whether the result is permanent. Although several years of complete freedom from asthma have elapsed in a few instances, in other patients not "cured," whose cases are therefore not included in this paper, it was found that with a return to the old environment asthma recurred. In contradiction to the idea that such a gloomy outlook applies to all such cases is the story not uncommonly given that patients "used to be" sensitive to a cat, to feathers or to some particular dust, but find that now such an intolerance does not exist. It is unfortunate that more of the "cured" patients have not returned to the environment which caused their trouble so as to provide the data necessary to test their "cure."

The intrinsic group is even more difficult of analysis. It appears that vaccines cause a "cure" in some cases and that potassium iodide will relieve a patient with bronchitis markedly and perhaps permanently. On the other hand, both vaccines and iodides have been given to countless other patients without such results.

The operative treatment of patients with lesions in the nose and throat, the removal of bad teeth and other procedures are additional measures which will "cure" some patients, but in the vast majority of cases the effect is only temporary. A detailed study of the relation of the nose and throat to asthma is in preparation.

Throughout this paper, "cure" by "hygiene" is frequently mentioned. This shows that the principles of general medical care with attention to the patient as a whole are an important part of the management. When such simple measures as the relief of constipation, the readjustment of diet (without any attempt to omit or add a particular food) and the institution of good habits of rest and exercise can bring such excellent results in the proper cases, it is apparent that any physician with experience and common sense can do a great deal for asthmatic patients without the use of complicated apparatus or the making of tests which are often so hard to interpret.

"Cures" by "hygiene" are especially important in children. The chart shows the general distribution of "H's" but also a concentration of "H's" among those in whom asthma began at an early age and who are still children. Hygiene presumably does little more than allow nature to cure the disease, and then the child is said to have "outgrown" his asthma. This subject is so important that the end-results in all those who developed asthma at an early age have been tabulated.

Table 3 shows the results in the entire series without regard to the type of asthma or the method of treatment. There are 351 patients in whom asthma began before the age of 12. Of these, 288 are still under 20 years of age, while 123 are over 20. Patients who are "cured" and "greatly improved" (a designation which includes several patients who have been free from asthma for a year, but who are not yet eligible for the "cured" group) form 66 per cent of the 228 patients who are under 20 years of age, while good results among those now past 20 occur in only 38 per cent of the 123 cases.

Of more importance than the present age, however, is the age at which these children applied for treatment (table3). Figures show that of the patients whose cases were studied before the age of 12, 33 per cent are "cured" and another 32 per cent are "greatly improved," a total of 65 per cent; whereas if study was postponed until the patients were older, the figures are reduced to 20 and 28 per cent, or a total of 48 per cent. The age of the patients when their cases are first studied, however, is roughly parallel to the exact age of onset, since 110 (61 per cent)

of the patients seen before the age of 12, developed asthma before the age of 5, while of those seen after 12, the age of the onset of the disease is a little later, since the attacks began in only 88 (48 per cent) before the age of 5.

These figures bring out the marked tendency of patients to outgrow asthma when it begins in infancy; they also show that the prognosis is better when the onset is earlier. As the highest figure is only 66 per cent, it is evident that an attitude which is too complacent is dangerous, and that in spite of the favorable outlook, all reasonable efforts to control the condition must be made in every case.

Is asthma which is "outgrown" different from asthma which is "cured" by hygiene, and what factors have these cases in common with

TABLE 3.—*Asthmatic Patients with an Onset Before 12 Years of Age*

A: Present Status According to Present Age

End-Results	Under 20 Years		Over 20 Years		Total	
	Number	Per Cent	Number	Per Cent	Number	Per Cent
"Cured"	80	35	11	9	91	26
Greatly improved	70	31	36	29	106	30
Improved	54	24	41	33	95	27
Same or worse	19	8	22	18	41	12
Dead	5	2	13	11	18	5
Total	228	100	123	100	351	100

B: Present Status According to Age When First Studied

End-Results	Studied Before 12 Years		Not Studied Before 12 Years		Total	
	Number	Per Cent	Number	Per Cent	Number	Per Cent
"Cured"	55	33	36	20	91	26
Greatly improved	54	32	52	28	106	30
Improved	39	23	56	31	95	27
Same or worse	17		24		41	
Dead	3	12	15	21	18	17
Total	168	100	183	100	351	100

the occasional true cures in adults? Such thoughts as that of the changes at puberty or of the influence of endocrine glands are suggested, but there is no evidence to substantiate any one of them. In any case, it is evident that the chief mechanism of asthma is immunologic rather than physiologic or anatomic, but what controls and changes this immunologic reaction remains unknown.

CONCLUSIONS

The foregoing study is based on an analysis of 213 cases in which the patients have been relieved of their asthma for over two years. These 213 patients are merely a part of the total of 1,074 patients on whom the end-results are known, as previously reported.

The most common method of "cure" is by the elimination of or the escape from the particular external substance to which the patient is hypersensitive and which, as the exciting cause (the trigger mechanism),

starts the asthmatic attack. In certain cases, especially in children, the removal of this trigger mechanism can result in a permanent cure, but in most of the cases, the extent of "cure" in asthma cannot be determined.

Clinical "cure," including absence of symptoms even in the presence of the supposedly offending substance, can exist in spite of the fact that reactions to skin tests are still positive; consequently, the thought that positive reactions may represent past history rather than present illness is presented.

Apparent "cures" following changes in environment may be permanent in some cases.

The removal of foci of infection leads to clinical "cure" in a few cases, although similar procedures do not help other patients who may later be "cured" by other means.

Faulty hygiene, often the result of asthma, may apparently be associated with the cause of asthma in a considerable number of cases, so that treatment based on the principles of common sense medicine is always important, especially as children tend to outgrow asthma, and this tendency can be assisted by good management begun as early as possible.

The evidence at hand fails to suggest any definite explanation of the asthmatic state, except that the basis is immunologic rather than physiologic or anatomic.

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EXPERIMENTAL ANEMIA PRODUCED BY CLOSTRIDIUM WELCHII

CHEMICAL ANALYSIS OF THE BLOOD *

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In a preceding paper,[1] the general problem of the significance of the anaerobic intestinal bacteria in relation to human disease was presented, and experimental studies of these microbes, more especially of those belonging to the species *Clostridium welchii,* were described. A toxic anemia of severe grade was induced by inoculation of these organisms.

A comparison of the results of chemical analyses of the blood of these experimental animals with similar results on control normal animals, and especially with analogous results obtained in clinical diseases of man, is our purpose in the present communication. The difficulty of applying conceptions derived from animal experimentation at once in clinical medicine is keenly appreciated. Nevertheless, living things have much in common, and the results of animal experimentation have already proved of value in the study of anemia.

In the present study, attention has been devoted especially to that group of experimental animals in which the most striking changes were observed in the blood, namely, the animals inoculated with toxic strains of *C. welchii.* Quantitative chemical analyses of the blood were made in conjunction with other observations on these same animals, recorded in the previous paper. By reference to the protocols there presented, the chemical observations can be fitted into the more complete experimental record.

TECHNIC

Adult male rabbits were used exclusively, and only toxic strains of *C. welchii* were employed. Some of the animals were injected intramuscularly with eighteen hour cultures in anaerobic dextrose broth, in doses of from 0.5 to 1 cc. A severe grade of anemia was induced in these animals in five days. Toxic bacteria-free filtrates made by the method of Bull and Pritchett[2] and

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1. Patterson, Marjorie B., and Kast, Ludwig: Toxic Anemia Produced by Clostridium Welchii, Arch. Path. **5** (March) 1928.

2. Bull, Carroll G., and Pritchell, Ida W.: J. Exper. Med. **26**:867, 1917.

employing the broth formula of Kahn and Torrey[3] were injected intravenously into rabbits of a second group in initial doses of from 1 to 3 cc., followed in some instances by a second dose on the third or fourth day if further effect was desired.

In order to induce more chronic toxemia, rabbits were given repeated intramuscular injections of small quantities of whole culture or intravenous injections of toxic bacteria-free filtrates, the initial dose varying from 0.1 to 0.5 cc. Chemical analyses of the blood of these animals were made from time to time over a period of from one to two months. In conjunction with these observations, blood counts and estimation of hemoglobin by the Dare hemoglobinometer were carried out, usually just before obtaining the blood sample for chemical analyses. Data of these related examinations have been presented in the preceding paper.

In the chemical analyses of the blood, Newcomer's[4] method for hemoglobin as described by Myers[5] and Wong's procedure have been utilized, methods which have been found to be the most dependable by Lindsay, Rice and Selinger.[6] Figures for hemoglobin thus obtained are presented in the present communication. The Folin-Wu[7] method was employed for the blood sugar. For cholesterol, the procedure of Myers and Wardell with the modifications suggested by Baumann and Holly[8] was followed. Trichloracetic acid was used as the protein precipitant in estimations of the nonprotein nitrogen. The technic outlined by Bernheim[9] was adopted for the icterus index of the blood serum. With the exception of the icterus index, all analyses were made on oxalated whole blood drawn after an eighteen hour fast.

ANALYTIC RESULTS

The results of blood analyses on control normal rabbits are presented in table 1, together with the maximum, minimum and average values of the series. From sixteen to thirty control animals were examined to establish the average figure, except in the case of the icterus index, for which only six control determinations were made.

It is evident from the table that the control animals showed marked variations in concentration of the blood constituents studied. Nuzum, Osborne and Sansum[10] have shown that the character of the diet influences the level of nonprotein nitrogen and of urea nitrogen in the blood of rabbits. In monthly specimens from control rabbits over a period of

3. Kahn, M. C., and Torrey, J. C.: Proc. Soc. Exper. Biol. & Med. **22**:8, 1925.

4. Newcomer, H. S.: J. Biol. Chem. **37**:465, 1919.

5. Myers, Victor C.: Practical Chemical Analysis of Blood, St. Louis, C. V. Mosby Company, 1924.

6. Lindsay, J. W.; Rice, C. E., and Selinger, M. A.: J. Lab. & Clin. Med. **11**:737, 1926.

7. Folin, O., and Wu, H.: J. Biol. Chem. **41**:367, 1920.

8. Baumann, E. J., and Holly, O. M.: J. Biol. Chem. **55**:457, 1923.

9. Bernheim, A. R.: Icterus Index (A Quantitative Estimation of Bilirubinemia), J. A. M. A. **82**:291 (Jan. 26) 1924.

10. Nuzum, F. R.; Osborne, M., and Sansum, W. D.: Experimental Production of Hypertension, Arch. Int. Med. **35**:492 (April) 1925.

a year, these authors observed variations in urea nitrogen of the blood from 14 to 23 mg. and in nonprotein nitrogen from 32 to 38 mg. per hundred cubic centimeters. In experimental animals fed on a high protein diet for twelve months, they observed an increase in both nonprotein and urea nitrogen of more than 100 per cent above the level of the controls, and in these animals there were evident renal lesions. Horvath [11] has reported figures for urea nitrogen of the blood of rabbits on a diet of millet and cabbage, varying from 13 to 25 mg. per hundred cubic centimeters. In general, our rabbits received a diet of cabbage

TABLE 1.—*Analyses of Control Specimens of Rabbit Blood*

Rabbit	Hemoglobin, Gm. per 100 Cc.	Red Cells, Millions per C.mm.	Color Index	Nonprotein Nitrogen, Mg. per 100 Cc.	Urea Nitrogen, Mg. per 100 Cc.	Urea N. / N.P.N. Ratio	Amino-Acid Nitrogen, Mg. per 100 Cc.	Sugar, Mg. per 100 Cc.	Cholesterol, Mg. per 100 Cc.
3	12.7	6.28	0.62	44.2	13.5	0.30	9.1	295	79
4	13.4	4.74	0.87	39.1	16.4	0.42	8.7	136	70
5	11.8	6.82	0.53	36.7	....	....	...	...	62
6	12.5	7.02	0.55	41.3	....	....	...	166	67
7	10.8	6.76	0.66	46.4	....	....	...	172	58
8	9.2	5.20	0.53	37.0	14.0	0.37	8.5	150	68
9	13.4	7.38	0.56	36.2	10.7	0.29	10.3	135	91
33	7.6	7.44	0.31	37.5	15.8	0.42	6.5	...	69
58	11.2	5.84	0.58	40.8	12.3	0.30	...	115	68
59	11.8	5.84	0.62	42.3	20.2	0.47	...	108	72
60	10.9	5.84	0.56	43.3	16.9	0.39	...	130	87
61	11.3	4.50	0.67	45.4	15.9	0.35	...	109	86
62	12.7	6.62	0.59	42.3	10.4	0.24	...	109	60
63	12.8	6.62	0.59	39.4	20.2	0.51	...	120	57
64A	11.7	7.28	0.49	46.0	18.7	0.40	6.3	119	59
65	10.6	7.20	0.44	42.6	20.2	0.47	...	107	92
66	10.7	6.90	0.47	52.1	26.7	0.51	...	123	74
67	11.0	5.12	0.65	49.9	24.2	0.44	...	107	87
68	12.1	7.26	0.51	38.6	14.2	0.27	6.3	118	108
69	11.2	7.20	0.47	40.3	10.8	0.26	8.2	144	65
70	10.9	7.40	0.44	34.5	11.7	0.33	6.6	140	64
71	11.8	6.60	0.54	40.0	14.0	0.35	7.4	187	94
72	14.8	6.20	0.72	43.0	10.0	0.23	8.7	194	75
73	11.1	6.54	0.52	37.5	9.6	0.25	8.4	111	81
74	8.9	6.64	0.40	38.8	14.1	0.36	9.9	150	57
75	10.0	5.86	0.52	30.2	8.2	0.27	9.7	111	96
76	10.2	5.90	0.52	34.6	12.8	0.37	10.9	125	59
79	12.1	5.78	0.64	49.1	18.4	0.37	8.2	125	75
90	....	5.24	....	....	....	....	...	99	61
91	....	5.08	....	....	....	....	...	79	68
Maximum	14.8	7.44	0.87	52.1	26.7	0.47	10.9	295	108
Minimum	7.6	4.50	0.31	30.2	8.2	0.23	6.3	79	57
Average	11.4	6.37	0.55	41.3	15.1	0.35	8.3	132	73

supplemented occasionally with carrots, oats and hay. Animals subjected to acute intoxication experiments received cabbage exclusively, beginning two days before the first inoculation. In the twenty-five control observations on urea nitrogen shown in table 1, the value ranges from 8.2 to 26.7 with an average of 15.1 mg. per hundred cubic centimeters of blood. For normal human blood, Myers [12] gives from 10 to 15 mg. of urea nitrogen per hundred cubic centimeters, and Kast and Wardell [13] have

11. Horvath, A. A.: J. Biol. Chem. **68**:343, 1926.

12. Myers, V. C.: Physiol. Rev. **4**:274, 1924.

13. Kast, L., and Wardell, E. L.: Urea in Human Blood, Arch. Int. Med. **22**:581 (Nov.) 1918.

recognized 20 mg. per hundred cubic centimeters, as the upper normal limit for hospital patients. In respect to nonprotein nitrogen, the maximum variation in table 1 is from 30.2 to 52.1 mg. per hundred cubic centimeters. Only six rabbits gave figures above 45 mg. The hemoglobin content of the blood of these control rabbits varied from 7.6 to 14.8 Gm. with an average of 11.04 Gm. per hundred cubic centimeters.

The results of chemical analyses of the blood of rabbits recently infected by injection of whole broth cultures of *C. welchii* are set forth in table 2. Most striking is the decrease in the hemoglobin accompanied by a

TABLE 2.—*Biochemical Determinations Made on the Blood of Rabbits in Which an Acute Infection Was Produced with Whole Broth Cultures of (Groups 1 and 2) Strains of C. welchii*

Rabbit	Day of Treatment	Dose, Cc.	Hemoglobin, Gm. per 100 Cc.	Red Cells, Millions per C.mm.	Color Index	Nonprotein Nitrogen, Mg. per 100 Cc.	Urea Nitrogen, Mg. per 100 Cc.	Sugar, Mg. per 100 Cc.	Cholesterol, Mg. per 100 Cc.
64A	Normal	...	11.7	7.28	0.49	46.0	18.7	119	59
	1	0.5							
	3	None	8.9	3.78	0.72	42.8	14.7	147	44
	4	0.3							
	6	None	4.2	1.08	1.25	32.7	14.9	147	51
66	Normal	...	10.7	6.90	0.47	52.1	26.7	122	74
	1	0.5							
	3	None	8.4	3.80	0.67	45.8	....	158	60
	5	None	3.3	1.58	0.66	62.5	29.5	146	91
65	Normal	...	10.6	7.20	0.44	42.6	20.2	107	92
	1	0.5							
	3	None	7.9	3.76	0.64	39.7	....	125	62
	4	0.3							
	6	None	5.1	3.60	0.43	39.1	25.6	76	87
	8	None	4.9	3.74	0.40	54.0	30.6	125	97
63	Normal	...	12.8	6.62	0.59	39.4	20.2	129	57
	1	0.5							
	3	None	7.7	5.06	0.47	35.2	....	150	54
	4	0.3							
	6	None	6.5	4.40	0.44	45.1	19.0	136	82
	8	None	6.2	4.40	0.43	48.7	19.9	115	77
68	Normal	...	12.1	7.26	0.51	38.6	14.2	118	108
	1	0.5							
	3	None	8.7	5.40	0.49	43.3	15.5	164	75

somewhat more marked diminution in the number of circulating erythrocytes. In specimens of blood obtained by puncture of the ear vein of rabbits, Bushnell and Bangs [14] found an average normal erythrocyte count of 5,988,500 per cubic millimeter. Extreme variations from 4,322,000 to 6,976,000 were observed in a series of hourly observations on one normal rabbit. A red cell count below 4,307,000 per cubic millimeter in rabbits is considered pathologic by these authors. Control counts made by a similar procedure on rabbits used in the present investigation gave an average figure of 6,250,000 with extremes of 9,020,000 and 4,500,000 per cubic millimeter in sixty-eight observations. The figure for the rabbit is higher than the erythrocyte count in human

14. Bushnell, L. D., and Bangs, E. F.: J. Infect. Dis. **39**:291, 1926.

blood. The hemoglobin content, however, is in general lower in rabbits than in man, so that the color index calculated on the same basis as in human blood is normally less than unity in these animals, varying from 0.31 to 0.87, with an average of 0.55 in the control observations. In the experimental animals, erythrocyte counts as low as 1,080,000 and 1,580,000 were observed, with a reduction of the hemoglobin to 4.2 and 3.3 Gm. per hundred cubic centimeters, respectively, and a rise in the color index as high as 1.25 in the first instance (rabbit 64 A). These striking effects on the erythrocytes have been set forth in the preceding paper.[1] It is, however, necessary to recall them here, because of the relation of changed cell concentration to the concentration of other constituents of the blood resulting from uneven distribution of these constituents in the cells and the plasma. The other analytic results are less striking on first inspection. The nonprotein nitrogen tended to diminish in the first few days, in all except rabbit 68. This change would appear to depend on the reduction in the number of erythrocytes, for Folin and Berglund [15] have demonstrated a greater concentration of nonprotein nitrogen in the corpuscles as compared with the plasma of blood, the difference being due to an excess of rest nitrogen in the cells. On the other hand, urea nitrogen is almost evenly distributed in the substance of corpuscles and plasma, and its concentration in whole blood is influenced little by changes in the number or volume of the cells. Folin,[16] discussing nonprotein nitrogen of human blood, states that, under normal conditions, the urea nitrogen forms from 35 to 55 per cent of the nonprotein nitrogen, most frequently from 40 to 50 per cent.

If one assumes, therefore, that the blood of rabbit 64 A before inoculation contained equal volumes of plasma and corpuscles and that $\frac{2}{5}$ of the nonprotein nitrogen, or 18 mg., was in the plasma and $\frac{3}{5}$, or 28 mg., in the corpuscles, it would appear that reduction of the corpuscles to 3,780,000 per cubic millimeter caused the plasma to constitute $\frac{3}{4}$ of the blood volume and the corpuscles $\frac{1}{4}$. Of the observed nonprotein nitrogen, namely, 42.8 mg. per hundred cubic centimeters, the indicated partition between plasma and cells would be in the ratio of $\frac{2}{5} \times \frac{3}{4} : \frac{3}{5} \times \frac{1}{4}$ or $\frac{6}{20} : \frac{3}{20}$ or 2:1. Two thirds of the observed 42.8 mg., or 29.5 mg., would therefore represent the nonprotein nitrogen of the plasma at this time, a distinct increase over the normal figure of 18 mg. before inoculation. In the same way, the observation on the sixth day in rabbit 64 A, namely 33.7 mg. nonprotein nitrogen per hundred cubic centimeters of blood, requires elucidation. At this time, the corpuscles numbered 1,080,000 per cubic millimeter, or approximately $\frac{10}{67}$ of their number before inoculation, so that they now made up about $\frac{1}{13}$ of

15. Folin, O., and Berglund, H.: J. Biol. Chem. **51**:418, 1922.
16. Folin, O.: Physiol. Rev. **2**:460, 1922.

the volume of the blood. The observed nonprotein nitrogen, on this assumption, would be partitioned between plasma and corpuscles in the ratio of $\frac{12}{13} \times \frac{2}{5} : \frac{1}{13} \times \frac{3}{5}$ or $\frac{24}{65}$ or 8:1. Eight ninths of the observed nonprotein nitrogen (33.7), or 30 mg., would represent the nonprotein nitrogen of the plasma at this time. While these calculations are not considered precisely accurate as representing actual conditions in the blood of this experimental animal, they, nevertheless, indicate definitely that the nonprotein nitrogen of the plasma has been increased during the course of the experimental disease. In some instances, as in rabbits 66, 65, 63 and 68, the nonprotein nitrogen of even the whole blood rose above the level observed before inoculation. It would, therefore, appear that the acute infection with *C. welchii* has caused an increased accumulation of intermediate nitrogenous catabolic substances in the blood, not yet transformed into the more normal excretory products such as urea.

The figures for urea nitrogen in this group of rabbits with acute infection are extremely variable. Without data concerning the urinary urea, one hesitates to interpret the figures for blood urea. However, it would appear that injury to the kidney may have been responsible for a moderate retention of urea in some of these animals, as for example in rabbit 65.

Bacteria-free toxic filtrates of cultures of *C. welchii* were injected into six rabbits so as to cause severe acute disturbances. The results of chemical analyses of the blood of these animals are shown in table 3. Here also the most striking feature is the marked diminution in the number of circulating erythrocytes and the corresponding but in general somewhat less marked reduction of hemoglobin in the blood. The consequent rise in the color index would appear to be a fairly characteristic feature of this toxic anemia. In these six animals, the nonprotein nitrogen acted much as in the preceding group, illustrated in table 2, again indicating an accumulation in the blood of intermediate nonprotein products of nitrogen catabolism. Rabbit 75 behaved in an exceptional manner, the marked retention of nonprotein nitrogen, of urea, of sugar and of cholesterol indicating severe impairment of renal function. This animal was killed on the fifth day, and histologic examination of the kidneys revealed an acute hemorrhagic and hemoglobinuric nephritis, distinctly more severe than the morphologic changes in the other animals of this group studied in the same way.

Milder, chronic infections with *C. welchii* were induced in six rabbits by the repeated injection of small doses of whole broth cultures of *C. welchii* over a period of from twenty-nine to fifty days. The results of blood analyses on these animals are shown in table 4. The reduction in erythrocytes and in hemoglobin was less in these animals, and after three

or four weeks, in spite of continued inoculations, there was a tendency toward restoration to normal. The chemical analyses were begun early in the third week of the treatment. Except for hemoglobin and red blood cells, the figures remain within the limits of observations on normal control animals. These observations are in accord with other evidence of an acquired immunity in these animals.

Toxic, bacteria-free filtrates of cultures of *C. welchii* were injected in divided doses into six rabbits over periods of from forty-eight to

TABLE 3.—*Biochemical Determinations Made on the Blood of Rabbits in Which an Acute Severe Toxemia Was Induced with Toxic Filtrates of (Groups 1 and 2) Strains of C. welchii*

Rabbit	Day of Treatment	Dose, Cc.	Hemoglobin, Gm. per 100 Cc.	Red Cells, Millions per C.mm.	Color Index	Nonprotein Nitrogen, Mg. per 100 Cc.	Urea Nitrogen, Mg. per 100 Cc.	Sugar, Mg. per 100 Cc.	Cholesterol, Mg. per 100 Cc.
71	Normal	...	11.8	6.60	0.54	40.0	14.0	187	94
	1	1.0							
	3	1.5	7.3	3.82	0.57	50.0	13.0	142	76
	4	2.0							
	6	None	5.2	2.44	0.64	43.5	10.5	162	53
74	Normal	...	8.9	6.64	0.40	38.8	14.1	150	57
	1	2.5							
	3	2.5	3.0	2.14	0.42	31.4	13.2	174	63
	4	3.0							
	5	None	4.1	1.60	0.78	53.6	14.5	159	128
75	Normal	...	10.0	5.86	0.52	30.2	8.2	111	96
	1	2.0							
	3	None	5.4	2.80	0.59	103.4	71.3	157	93
	4	2.0							
	5	None	4.4	1.80	0.72	156.2	106.0	170	153
76	Normal	...	10.2	5.90	0.52	34.6	12.8	125	59
	1	2.5							
	3	3.0	6.2	2.88	0.67	42.0	14.7	133	133
	4	3.0							
	5	None	5.3	2.00	0.80	37.0	14.8	170	107
28	Normal	...	....	5.02					
	1	2.0							
	2	2.0							
	3	3.0							
	5	None	4.7	2.58	0.56	52.3	19.1	...	107
29	Normal	...	....	6.00					
	1	3.0							
	2	3.0							
	3	5.0							
	5	5.0							
	6	None	5.8	3.22	0.54	58.5	19.3	...	86

seventy-eight days in the attempt to induce a chronic toxic anemia. Chemical analyses of the blood were undertaken only at the end of each experiment, on killing the animal, at a time when the earlier reduction of hemoglobin and of red blood cells had been partly restored toward normal. The results are shown in table 5. The figure for urea nitrogen remained below 20 mg. in all except rabbit 17, whereas the amount of nonprotein nitrogen tended to be high in all except rabbit 15. In the average figures for all six animals, the urea nitrogen constituted 31.8 per cent of the nonprotein nitrogen. Here again there is an indication

TABLE 4.—*Biochemical Determinations Made on the Blood of Rabbits in Which a Chronic Infection Was Produced with Whole Broth Cultures of (Groups 1 and 2) Strains of C. welchii*

Rabbit	Day of Treatment	Dose, Cc.	Hemoglobin, Gm. per 100 Cc.	Red Cells, Millions per C.mm.	Color Index	Nonprotein Nitrogen, Mg. per 100 Cc.	Urea Nitrogen, Mg. per 100 Cc.	Sugar, Mg. per 100 Cc.	Cholesterol, Mg. per 100 Cc.
58	Normal	...	11.2	5.84	0.58	40.8	12.3	115	68
	1	0.3							
	3	0.3							
	7	0.3							
	9	0.2							
	13	0.2							
	15	None	10.2	4.04	0.77	37.7	8.8	141	65
	20	0.2							
	22	None	9.4	4.73	0.60	27.3	10.6	122	78
	28	0.5							
	30	None	8.2	4.78	0.53	35.2	....	96	72
	31	0.5							
	36	0.5	7.4	3.76	0.60	31.6	8.3	122	78
	43	1.0	9.6	5.48	0.48	46.8	17.8	103	61
	50	None	9.0	5.30	0.51	34.5	8.9	161	66
59	Normal	...	11.2	7.20	0.47	40.3	10.8	144	65
	1	0.3							
	9	0.3							
	12	0.3							
	15	None	11.1	4.62	0.73	31.4	9.1	111	86
	22	0.5	9.3	5.08	0.57	51.7	10.7	166	65
	24	0.5							
	29	None	9.7	5.26	0.56	32.0	8.3	147	80
25	Normal	...	....	5.96					
	37	4.0	9.8	5.26	0.57	35.2	16.0	...	73
60	Normal	...	10.9	5.84	0.56	43.3	16.9	130	87
	1	0.1							
	3	0.1							
	6	0.1							
	15	None	6.4	3.20	0.60	42.8	14.8	86	59
	20	0.1							
	22	None	6.7	4.18	0.50	27.9	10.4	132	67
	28	0.2							
	30	None	7.0	4.56	0.46	42.6	....	56	82
	31	0.2							
	36	None	11.1	4.02	0.85	37.2	7.8	115	102
61	Normal	...	11.3	4.50	0.76	45.4	15.9	109	86
	1	0.1							
	3	0.1							
	6	0.1							
	13	0.1							
	16	None	7.2	3.06	0.73	33.8	10.2	144	83
	20	0.1							
	22	None	6.8	4.13	0.50	32.9	10.1	167	82
	28	0.2							
	30	None	8.3	5.64	0.44	33.8	....	106	114
	31	0.3							
	36	0.5	7.8	5.34	0.44	27.6	8.2	120	95
	43	1.0	8.5	4.46	0.59	45.1	15.7	107	92
	50	None	8.2	4.76	0.53	31.7	9.5	220	68
70	Normal	...	10.9	7.40	0.44	34.5	11.7	140	64
	1	0.1							
	9	0.1							
	12	0.1							
	15	None	8.3	3.40	0.73	31.8	10.0	99	117
	22	0.3	8.6	3.92	0.66	42.7	8.7	122	64
	29	None	10.2	5.04	0.62	65.8	33.1	109	90

of relative increase in nonprotein nitrogen other than urea, which is in accord with the observations on the more severe acute intoxications in rabbits.

Kahn and Barsky [17] have recorded figures for some of the blood constituents in three cases of pernicious anemia. The figures for nonprotein nitrogen ranged from 29.3 to 38.5 mg. per hundred cubic centimeters; for urea nitrogen, from 18.4 to 20.1 mg. In all three cases, the urea nitrogen formed more than one half of the nonprotein nitrogen, which might be expected in conditions in which the blood cells are diminished. Gettler and Lindeman [18] have presented data on chemical analyses of blood in thirty-two cases of pernicious anemia, including eighty-seven complete blood analyses. They found the nonprotein nitrogen of the blood increased above the normal limits (25 to 40 mg.) in 48 per cent of their cases, an amount too large to be accidental. Urea

TABLE 5.—*Biochemical Determinations Made on the Blood of Rabbits After Chronic Intoxications Had Been Produced with Toxic Filtrates of (Groups 1 and 2) Strains of C. welchii*

Rabbit	Day of Treatment	Total Toxin Given, Cc.	Hemoglobin, Gm. per 100 Cc.	Red Cells, Millions per C.mm.	Color Index	Nonprotein Nitrogen, Mg. per 100 Cc.	Urea Nitrogen, Mg. per 100 Cc.	Sugar, Mg. per 100 Cc.	Cholesterol, Mg. per 100 Cc.
12	73	37.5	8.6	4.62	0.56	49.5	19.0	...	98
22	47	27.25	6.0	5.44	0.33	50.0	16.4	...	73
15	50	33.4	8.9	4.98	0.55	34.6	19.2	176	61
21	49	46.5	11.0	4.98	0.68	97.4	11.7	...	92
17	78	32.4	11.6	5.40	0.65	72.1	38.1	...	81
20	48	34.5	9.1	5.32	0.51	73.4	18.3	...	83

nitrogen also tended to be high, but in only 18 per cent of the cases was it above the normal limit of 20 mg. They were inclined to ascribe these relations to the decreased amount of circulating blood and diminished power of oxidation within the cells. The observation of analogous changes in the blood of our rabbits after intoxication with products of *C. welchii* suggests that the increase of nonprotein nitrogen in patients with pernicious anemia as well as in these experimental animals may be due to exaggerated protein catabolism associated with a toxemia; however, they are not necessarily of the same causation.

Table 6 presents the results of analyses for amino-nitrogen as determined by Folin's [19] colorimetric method; these were, on the average, slightly higher (0.5 mg.) than those obtained by the gasometric pro-

17. Kahn, M., and Barsky, J.: Chemistry of Pernicious Anemia, Arch. Int. Med. **23**:334 (March) 1919.

18. Gettler, A. O., and Lindeman, E.: Blood Chemistry of Pernicious Anemia, Arch. Int. Med. **26**:453 (Oct.) 1920.

19. Folin, O.: J. Biol. Chem. **51**:374, 1922.

cedure of Van Slyke. Greene, Sandiford and Ross [20] have reported data on the amino-acid nitrogen of human blood in health and in a variety of pathologic conditions. In twenty normal specimens, the figures obtained were, maximum 8, minimum 5.1 and average 6.38 mg. per hundred cubic centimeters. In the blood of twelve young men after fasting one night, Berglund [21] found an average amino-acid nitrogen of 6.4 mg. per hundred cubic centimeters. The figures for the blood of control rabbits

TABLE 6.—*Amino-Acid Nitrogen of Blood in Acute and Chronic Intoxications*

Rabbit	Day of Treatment	Inoculum	Amino-Acid Nitrogen, Mg. per 100 Cc.
		Acute Intoxications	
64A	Normal	Whole broth culture	6.3
	3		7.0
	6		6.8
68	Normal	Whole broth culture	6.3
	3		8.3
71	Normal	Toxin	7.4
	3		9.0
	6		8.6
74	Normal	Toxin	9.9
	3		8.8
	5		9.2
75	Normal	Toxin	9.7
	3		8.8
	5		8.0
76	Normal	Toxin	10.9
	3		8.0
	5		8.4
		Chronic Intoxications	
69	Normal	Whole broth culture	8.2
	15		7.9
	22		7.8
	29		7.8
70	Normal	Whole broth culture	6.6
	15		6.8
	22		8.2
	29		8.7
12	73*	Toxin	8.0
22	47*	Toxin	9.2
15	50*	Toxin	7.0
21	40*	Toxin	8.2
17	78*	Toxin	9.6
20	48*	Toxin	10.1

* Termination of treatment.

in our table 1 are somewhat higher, namely: maximum, 10.9; minimum, 6.3; average, 8.3 mg. per hundred cubic centimeters. Greene, Sandiford and Ross,[20] in their exhaustive study of amino-nitrogen of the blood in disease, present figures for six cases of anemia (the type of anemia not stated) ranging from 5.1 to 8, with an average of 6.38 mg. per hundred cubic centimeters, identical with their average figure for twenty

20. Greene, C. H.; Sandiford, K., and Ross, H.: J. Biol. Chem. **58**:845, 1923-1924.

21. Berglund, H.: Nitrogen Retention in Chronic Interstitial Nephritis, J. A. M. A. **79**:1375 (Oct. 21) 1922.

normal specimens of blood. Gettler and Lindeman found abnormally high figures for amino-nitrogen in pernicious anemia, from 9 to 40 mg. per hundred cubic centimeters, as determined by Van Slyke's method, at least four times the normal in some cases. In table 6 are presented figures of the amino-nitrogen in the blood of rabbits with experimental anemia induced by injection of cultures or products of *C. welchii*. The variations are all within the normal limits for rabbits, as indicated in table 1. In this respect, these rabbits appear to be distinctly different from the patients with pernicious anemia studied by Gettler and Lindeman.

The control specimens of blood showed the greatest variations in the sugar content. These specimens were obtained about eighteen hours after feeding, but, even so, the animals cannot be regarded as in a postabsorptive state. For this purpose, Baumann and Holly [8] found it necessary to allow a fast of forty-eight hours in rabbits. The figures recorded in tables 2 and 3 show a moderate increase in blood sugar in most of the rabbits after the first injection, with a tendency to decrease subsequently in spite of further inoculations. These changes resemble the alterations in blood sugar observed in acute infections in man. They are not in the same category as the observations of Zeckwer and Goodell,[22] who observed rapidly developing hyperglycemia in rabbits after intravenous injection of killed bacteria, with return to normal in a few hours. Such rapid fluctuations in our animals would have escaped detection.

The blood cholesterol of thirty control rabbits shown in table 1 averaged 73 mg. per hundred cubic centimeters, near the figure (67 mg.) reported by Baumann and Holly [8] as the average of their observations in seventeen control rabbits. In our series, four rabbits had more than 90 mg. of cholesterol per hundred cubic centimeters of blood, and one animal had 108 mg. These specimens were obtained by heart puncture during brief ether anesthesia, and it is believed that the anesthesia could not have influenced the cholesterol observations, as Mahler [23] found the blood cholesterol to be significantly altered only after from fifteen to twenty minutes of deep anesthesia.

The cholesterol of human blood is much reduced during acute bacterial infections. Chauffard [24] has observed reduction of the cholesterol content of the suprarenals in pulmonary tuberculosis, tuberculous meningitis, pneumonia, typhoid fever and septicemia. In all types of anemia in man, the blood cholesterol is diminished. As the patient

22. Zeckwer, I. T., and Goodell, H.: J. Exper. Med. **42**:43, 1925.

23. Mahler, A.: J. Biol. Chem. **69**:653, 1926.

24. Chauffard, quoted by Campbell, J. M. H.: Quart. J. Med. **18**:393, 1924-1925.

recovers from the anemia or from the infection, the cholesterol increases. Horiuchi [25] produced experimental anemia in rabbits on a fat-free diet by repeated withdrawal of from 10 to 15 cc. of blood over a period of from five to seventeen days. In these animals, he observed an initial slight decrease of blood cholesterol followed by an increase to two or three times the normal control. Of our nine rabbits recorded in tables 2 and 3, one animal (no. 71) showed a progressive decrease of blood cholesterol, but in the others the initial decrease was followed by a subsequent increase. The decrease here was greater than that observed by Horiuchi, possibly because a more severe anemia was produced. The subsequent increase was, however, less than that described by him, and in no instance did the blood show evidence of a gross lipemia. In the rabbits subjected to a more prolonged mild intoxication, as recorded in table 4, the decrease in cholesterol also occurred, followed by a more definite indication of the rise in blood cholesterol in the later period of treatment. The evident immunity acquired by rabbits during administration of whole broth cultures or of toxic filtrates of *C. welchii* for a period of twenty-five days or longer, as shown by Patterson and Kast [1] in a previous paper, may be correlated with this rise in the blood cholesterol.

The icterus index of rabbit's blood is much lower than that of human blood, the latter ranging from 4 to 6 by Bernheim's technic. In six control rabbits, the icterus index ranged from 1.5 to 2.2 with an average of 1.95. In the experimental animals, particularly in those subjected to severe acute intoxication, the blood serum was often tinged with hemoglobin because of the violent hemolysis *in vivo*, and an estimation of the icterus index could not be made. The observations made at other times are recorded in table 7. Here it will be seen that the icterus index was found always to be above the upper extreme for the controls except in one observation made on rabbit 10 on the fifty-second experimental day. The highest value was 12.5 in rabbit 84 on the fifth day of treatment. Bernheim [9] has found that the icterus index varies from 6.5 to 12.5 with an average of 10 in pernicious anemia of man, while in secondary anemia her figures ranged from 2.3 to 3.9 with an average of 3.3. Taking into account the normally lower icterus index of the rabbit, the proportionate increase from 1.5 to 6 times the control figure in these experimental animals would appear to correspond closely with the elevation of the icterus index observed by Bernheim in human pernicious anemia. The phenomenon is not, however, a specific reaction to *C. welchii* in a bacteriologic sense, for a similar, if less marked, rise in the icterus index was observed in rabbits 87 and 71, which were treated with cultures of other intestinal anaerobes.

25. Horiuchi, Y.: J. Biol. Chem. **44**:347, 1920.

TABLE 7.—*Determination of the Icterus Index in the Blood of Rabbits in Which Acute and Chronic Intoxications Had Been Produced with C. welchii*

Rabbit	Day of Treatment	Inoculum	Icterus Index
		Acute Intoxications	
28	5	Toxin	3.0
29	6	Toxin	3.8
82	5	Toxin	8.0
83	5	Toxin	2.8
84	5	Toxin	12.5
86	6	Toxin	4.5
87	6	Toxin	6.5
		Chronic Intoxications	
17	46	Toxin	2.4
20	46	Toxin	2.6
15	49	Toxin	3.0
21	49	Toxin	3.1
12	42	Toxin	4.5
22	48	Toxin	2.6
23	28	Toxin	5.3
10	52	Whole broth culture	1.7
18	83	Whole broth culture	3.8
25	33	Whole broth culture	4.4
19	73	Whole broth culture	3.5
		Control Rabbits	
13	87	C. bifermentans culture	3.5
14	71	C. sporogenes culture	2.7

TABLE 8.—*Urobilin Excretion in Rabbits in Which Acute and Chronic Intoxications Had Been Produced with C. welchii*

Rabbit	Day of Treatment	Inoculum	Urobilin, Mg. per 100 Cc.
		Acute Intoxications	
32	7	Whole broth culture	94.0
24	5	Whole broth culture	153.0
64A	5	Whole broth culture	Trace
	6		188.0
66	5	Whole broth culture	188.0
7	6	Whole broth culture	189.0
68	3	Whole broth culture	Trace
	4		23.5
	5		23.5
	6		62.6
28	5	Toxin	Trace
27	5	Toxin	189.0
29	6	Toxin	710.4
71	3	Toxin	Trace
	6		11.1
74	5	Toxin	Trace
76	3	Toxin	Trace
	5		Trace
82	6	Toxin	47.0
86	6	Toxin	Trace
87	6	Toxin	12.0
		Chronic Intoxications	
21	49	Toxin	Trace
23	31	Toxin	11.8
11	63	Whole broth culture	47.6
18	83	Whole broth culture	94.0
19	4	Whole broth culture	88.17
	6		176.35
	19		118.1
61	29	Whole broth culture	Trace
69	15	Whole broth culture	Trace
70	29	Whole broth culture	Trace
		Control Rabbit	
6	20	C. bifermentans culture	47.25

An estimate of the rate of destruction of red blood cells can also be obtained from the urobilin output in the urine. This was determined by the method of Elman and McMaster [26] at intervals during the experimental treatment, on catheterized specimens of urine obtained from several of the rabbits and on urine removed from the bladder at autopsy on the same animals as well as on a larger number of others. In table 8, in which these values are presented, the latest experimental day on which an analysis is recorded is the day of death of the animal in each instance, and the value recorded for that day was found on analysis of the urine in the bladder at autopsy. Amounts of less than 3 mg. of urobilin per hundred cubic centimeters are recorded as "trace." The enormous variations shown in table 8 appear to be definitely correlated with variations in the process of erythrocyte destruction and apparently a more accurate measure of this than is the icterus index. Thus, rabbit 28 had a trace of urobilin in the urine and an icterus index of 3 on the fifth day of treatment, while rabbit 29 had 710 mg. of urobilin per hundred cubic centimeters of urine with an icterus index of 3.8 on the sixth experimental day. Evidently the icterus index is a measure of pigments circulating in the blood, while the urobilin figures represent pigments of hemolytic origin excreted through the bile ducts, transformed in the intestine, resorbed and excreted in the urine. The latter mode of disposition of the pigment products of hemolysis appears to be the chief normal mechanism and to be capable of extremely wide adaptation to the demands made on it by excessive blood destruction. In otherwise normal animals subjected to experimental hemolytic intoxication, the urobilin excretion would appear the better measure of this particular effect.

SUMMARY

1. Acute intoxication of rabbits with the products of *Clostridium welchii* resulted in a marked reduction in hemoglobin and an even greater reduction in the number of erythrocytes of the circulating blood.

2. In most of these animals there was an increase in the nonprotein nitrogen of the blood plasma without a corresponding increase in urea nitrogen, and hence a diminished ratio of the latter to the former. This alteration appears to be a result of abnormal protein catabolism associated with the intoxication.

3. The amino-nitrogen of the blood of the experimental animals, including those with severe acute as well as the more chronic intoxications, did not vary beyond the normal limits observed in control rabbits.

4. The blood sugar rose slightly in the acute intoxications, reaching its maximum about the third day and then subsiding in spite of further inoculations.

26. Elman, R., and McMaster, P. D.: J. Exper. Med. **41**:503, 1925.

5. The blood cholesterol tended to diminish in the early stages of the experimental intoxication and to increase subsequently, especially in the animals in which the erythrocytes and hemoglobin tended to return to normal.

6. The icterus index of the blood serum in the experimental animals was increased from 1.5 to 6 times the control figures. This change was most evident when rapid destruction of erythrocytes occurred.

7. The urobilin output in the urine varied from a trace to 710 mg. per hundred cubic centimeters, the large amounts being excreted after active destruction of blood.

8. Many of these changes resemble changes observed in anemia in man.

THE NONPROTEIN SULPHUR OF THE BLOOD IN CERTAIN PATHOLOGIC CONDITIONS*

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In this paper we have collected the results of a considerable number of observations made on the distribution of the nonprotein sulphur of the blood of normal persons and of persons suffering from various pathologic conditions of the kidneys, heart and liver.

Experimental evidence is available which indicates [1] that nonprotein sulphur exists in blood in the forms of at least three fractions, which appear to possess chemical properties similar to the several classes of sulphur compounds present in urine. Thus, there is present in blood a fraction designated as inorganic sulphate, which is precipitated by the addition of barium chloride to the deproteinized blood filtrate after suitable adjustment of the reaction of the latter. When the deproteinized blood filtrate is heated with acid, an additional precipitation with barium chloride results, and the difference between the value of the total sulphate so obtained and that of the inorganic sulphate is designated as ethereal sulphate. When the deproteinized blood filtrate is treated with a suitable oxidizing agent, certain unoxidized sulphur compounds contained therein are oxidized to sulphate and may be precipitated with barium chloride. The difference between the value for total nonprotein sulphur so obtained and total sulphates is designated as neutral sulphur.

The samples of blood used were obtained from the medical service of the New Orleans Charity Hospital. The analytic methods were those recently described by Denis and Reed.[1] The figures for nonprotein nitrogen were obtained by the method of Folin and Wu [2] and those for sodium chloride by the procedure of Whitehorn.[3]

The results of the examination of three samples of blood from normal persons (workers in the laboratory) and of thirty-one samples taken from patients in the hospital are given in table 1. In addition to the material contained in this table, we are also in possession of the

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1. Denis, W., and Reed, L.: J. Biol. Chem. **71**:191, 1926.
2. Folin, V., and Wu, H.: J. Biol. Chem. **38**:87, 1919.
3. Whitehorn, J. C.: J. Biol. Chem. **45**:449, 1920-1921.

TABLE 1.—*Nonprotein Sulphur in Whole Blood and Plasma*

Case	Laboratory Specimen	Mg. per 100 Cc. Blood: Nonprotein Nitrogen	Mg. per 100 Cc. Blood: Sodium Chloride	Mg. per 100 Cc. Blood: Inorganic Sulphate	Mg. per 100 Cc. Blood: Ethereal Sulphate	Mg. per 100 Cc. Blood: Neutral Sulphur	Mg. per 100 Cc. Plasma: Inorganic Sulphate	Mg. per 100 Cc. Plasma: Ethereal Sulphate	Mg. per 100 Cc. Plasma: Neutral Sulphur	Diagnosis
1	19	—	—	0.28	0.34	3.73	0.51	0.96	1.47	Normal
2	20	34	—	0.30	0.42	3.53	—	—	2.14	Normal
3	18	—	—	0.45	0.15	3.24	0.85	0.91	1.72	Normal
4	6	41	540	0.33	1.30	4.43	1.32	1.33	1.97	Nephritis, edema, Nov. 3, 1926
	9	39	583	1.98	1.09	4.03	1.94	0.76	1.98	Same patient as above, Jan. 28, 1927
5	17	41	506	0.34	0.96	3.49	0.66	0.66	2.57	Myocardial insufficiency, congestive heart failure, April 8, 1927
	14	30	486	0.56	0.60	3.19	1.12	0.35	2.02	Same patient as above, March 26, 1927
6	8	40	502	0.45	0.84	4.01	1.48	0.24	3.18	Subacute hemorrhagic nephritis
7	33	35	417	0.46	0.32	3.80	0.92	0.91	1.11	Myocardial insufficiency, ascites, congestive heart failure, jaundice
8	16	67	413	0.47	0.38	4.21	0.85	0.89	1.59	Cardiorenal disease, hypertension, edema
9	13	39	452	0.49	0.14	4.32	0.99	0.48	1.74	Asthmatic bronchitis, emphysema, chronic nephritis
10	23	26	509	0.53	0.15	4.00	1.07	0.48	1.30	Eclampsia, convulsive
11	31	32	472	0.54	0.32	3.39	1.08	0.56	0.95	Hyperplesia, arteriosclerosis, nephritis ?
12	22	35	484	0.55	0.54	4.59	1.06	0.31	3.30	Hypertension, arteriosclerosis, chronic nephritis
13	11	50	524	0.55	0.07	3.31	1.10	0.50	1.83	Alcoholic cirrhosis of the liver, jaundice
14	24	25	528	0.56	0.21	2.94	1.13	1.07	—	Edema, jaundice
15	26	37	505	0.59	0.31	3.64	1.18	0.82	1.7	Arteriosclerosis, chronic myocarditis, chronic nephritis
16	21	50	484	0.60	1.33	4.96	1.17	1.39	3.21	Cardiorenal disease, hypertension, arteriosclerosis
17	29	39	530	0.60	0.45	3.70	1.21	0.94	1.32	Hypertension, lesions of the central nervous system due to primary arteriosclerosis
18	32	41	492	0.63	0.29	5.94	1.25	0.99	1.91	Chronic nephritis, hypertension, arteriosclerosis
19	12	74	530	0.65	1.06	5.20	0.93	0.10	2.09	Jaundice, postoperative obstruction
20	35	52	445	0.76	0.48	4.10	1.83	0.47	2.43	Cardiorenal disease, ascites
21	25	80	403	1.09	1.15	5.01	1.61	3.31	2.24	Cardiorenal disease, hypertension, edema and ascites
22	27	82	410	1.68	0.10	4.69	3.62	0.73	0.80	Nephritis, hypertension, edema, syphilitic aortitis
23	T	63	644	2.55	0.58	6.23	1.56	1.45	1.27	Chronic and acute nephritis hypertension and anasarca, July 2, 1926
	5	51	780	5.32	2.54	8.99	3.60	1.62	3.27	Same patient as above July 26, 1926
24	36	58	494	2.88	2.04	4.17	3.00	1.72	3.83	Chronic nephritis, hypertension
25	2	48	540	3.08	1.07	2.99	2.75	1.61	1.92	Cardiorenal disease, hypertension, arteriosclerosis
26	30	60	525	4.37	0.40	3.08	4.37	0.63	3.04	Cardiorenal disease, hypertension, arteriosclerosis
27	3	44	491	4.72	0.93	3.51	4.53	0.68	2.27	Chronic nephritis, hypertension, edema, heart failure
28	28	91	554	4.80	0.11	4.13	4.80	0.51	3.26	Chronic nephritis, hypertension, edema
29	7	175	599	5.15	3.00	9.50	3.50	5.53	3.35	Chronic nephritis, uremia
30	BB	80	466	11.0	3.35	6.07	10.95	—	—	Chronic nephritis, congestive heart failure, edema
31	34	188	600	13.96	1.64	4.70	13.44	2.36	3.15	Cardiorenal disease, uremia, congestive heart failure

results obtained with the blood in a series of cases which in number exceeds those presented in the table. As, however, we were unable to obtain in these patients samples of blood of sufficient size for the determination of all of the nonprotein sulphur fractions in both whole blood and plasma, and as the result did not appear to present points of interest not already shown in the tabulated results, we have omitted the presentation of these incomplete analyses.

COMMENT

Within the past five years, several papers have been published on the subject of the concentration of the various nonprotein sulphur fractions of the blood of man. Thus, Meyer-Bisch [4] determined total (inorganic + ethereal) sulphate in the whole blood of persons suffering from a variety of pathologic conditions. His figures, however, are so much higher than ours that comparison is impossible. Kahn and Postmontier [5] published an executive series of analyses of the total sulphate and total nonprotein sulphur in normal and pathologic whole

TABLE 2.—*Results of Determinations of Nonprotein Sulphur Fractions of Normal Persons*

	Whole Blood	Plasma
Inorganic sulphate, mg. per 100 cc.	0.45	0.87
Ethereal sulphate, mg. per 100 cc.	0.35	0.67
Neutral sulphur, mg. per 100 cc.	3.80	1.95

blood, while Loeb and Benedict [6] made observations on the inorganic sulphate in the serum in a series of cases. In general, the values obtained in the two latter investigations lie within the general range found in our series.

As far as we are aware, analyses other than our own are not available for the nonprotein sulphur of both plasma and whole blood in pathologic conditions.

In a recent paper, two of us [7] placed on record the results of analyses of nine samples of human blood in which determinations were made of the nonprotein sulphur fractions in whole blood and in plasma of nine normal persons. An average of these results is given in table 2.

While the number of samples from which these average figures were obtained is, of course, too small to give values which might be considered to show the final limits of variation of these fractions under

4. Meyer-Bisch, R.: Bio-Chem. S. **101**:23, 1924.
5. Kahn, G., and Postmontier, R. S.: J. Lab. & Clin. Med. **10**:317, 1925.
6. Loeb, R. F., and Benedict, E. M.: J. Clin. Investigation **4**:33, 1927.
7. Denis, W., and Reed, L.: J. Biol. Chem. **73**:623, 1927.

normal conditions, they serve to indicate the general trend of distribution between plasma and corpuscles. Thus, it will be seen that the inorganic and ethereal sulphate fractions exist in largest amount in the plasma; in fact, if the corpuscles are considered as forming 50 per cent of the value of whole blood, it would appear from these figures that the corpuscles may be entirely devoid of inorganic and ethereal sulphate. On the other hand, the neutral sulphur fraction would appear to exist in largest amount, although not exclusively, in the corpuscles.

The results contained in table 1 indicate that there is no relation between the retention of nonprotein nitrogen and of inorganic sulphate, an observation not in accord with those of Loeb and Benedict [6] or of one of us.[8] Thus, case 30 with a nonprotein nitrogen of 60 mg., shows an inorganic sulphate content of 4.37 mg. in the whole blood, whereas case 25, with a nonprotein nitrogen of 80, shows an inorganic sulphur of 1.09. In a series of experiments [9] on dogs with uranium nephritis, it was found that the inorganic sulphur fraction of the blood could be strikingly affected by changes in the volume of urine excreted. Thus, in dogs with marked diuresis, the inorganic sulphate of the blood was so reduced that quantitative determination became impossible, whereas with reduction of the output of urine this fraction was much elevated over the normal value.

Most of the patients used in this work had nephritis or cardiorenal disease, and, on looking over the histories, we found that the use of diuretics apparently exerts a profound influence on the level of the inorganic sulphate fraction of blood. Thus, the specimens marked six and nine, respectively, were obtained from the same patient, who had nephritis with generalized edema. Specimen 6 (inorganic sulphate, 0.33 mg., nonprotein nitrogen, 39 mg.) was obtained just after treatment with merbaphen, the administration of which had caused marked diuresis, while specimen 9 (inorganic sulphate, 1.98 mg., nonprotein nitrogen, 41 mg.) was taken three months later when the patient had again become edematous, with marked reduction in the volume of urine excreted.

Determinations of chloride were made in this series because it seemed possible that fluctuations in the level of inorganic sulphate and of chlorides might run a parallel course. However, this is apparently not the case. As many of these patients had been on salt-free diets for varying lengths of time, it seems possible that different results might be obtained under other dietary conditions.

In normal human blood, the concentration of inorganic sulphate in plasma is usually about double that found in whole blood; as the concentration of this constituent rises, the concentration in the whole blood

8. Denis, W.: J. Biol. Chem. **49**:311, 1921.
9. Denis, W., and Reed, L.: J. Biol. Chem. **73**:41, 1927.

increases more rapidly than in the plasma, indicating, apparently, the diffusion of sulphate into the normally sulphate-free corpuscles. The result is that in patients showing marked retention the concentration of inorganic sulphate in whole blood and in plasma may be approximately equal. In this connection, it may not be out of place to call attention to the work of DeBoer,[10] who has demonstrated experimentally that at a concentration of carbon dioxide well within physiologic limits the corpuscles of the horse are capable of absorbing sodium sulphate added to blood.

The concentration of ethereal sulphate in normal human blood is, in the majority of specimens examined, considerably greater in plasma than in whole blood, although occasionally the reverse is true. The fluctuations in the figures for this fraction found in the specimen of pathologic blood do not in any way appear to bear a direct relation to the changes observed in the concentration of the inorganic sulphate or of the neutral sulphate fractions. As at present there is not direct evidence to indicate the origin or function of this fraction, speculation on the point would appear fruitless, and we therefore feel justified in merely placing the results on record.

In the normal person, the neutral sulphate fraction appears to be found in greatest concentration in the corpuscles. As shown by the higher values obtained in whole blood as compared to those found in the analysis of plasma, the same general relation appears to hold in the pathologic specimens examined; while with retention of inorganic sulphates concentration of neutral sulphate somewhat above the normal is sometimes found, this is not the rule. In two specimens (numbers 7 and 5), a greatly increased amount of the neutral sulphur fraction was found. Both of these specimens of blood were taken from patients who died a little later, and it is possible that in these cases oxidation within the corpuscles was progressing at a subnormal rate.

CASE HISTORIES IN ABSTRACT

In cases 1, 2 and 3, the subjects were normal young adult males. The histories of the rest of the cases in the order given in table 1 follow.

Case 4 (laboratory specimens numbers 6 and 9 in table 1).—S. P., a white swamp woodsman, aged 44, came to us with a generalized anasarca, which included puffiness about the eyes. He also complained of frequency of micturition, nocturia, cramps in the legs at night, vertigo, amblyopia, drowsiness and vomiting. The clinical picture suggested a nephritis of the type that causes retention of salt and water. Besides the evidence substantiating the complaints, pallor and pastiness, flabby musculature, carious infected roots of the teeth, chronic septic tonsillitis, slight enlargement of the heart and increase in the retromanubrial dulness, metallic valvular heart sounds, sinus tachycardia, an aortic systolic murmur, slight periph-

10. DeBoer, S.: J. Physiol. **51**:211, 1917.

eral sclerosis and an elevated blood pressure of 180 mm. of mercury systolic and 100 mm. of mercury diastolic were present.

The urine varied in specific gravity from 1.005 to 1.012 and contained from 2 to 30 per cent of moist albumin with hyaline and granular casts. The excretion of phenolsulphonphthalein was from 15 to 40 per cent in two hours. Chemical analyses of the blood revealed nonprotein nitrogen, 36 to 68 mg.; cholesterol 132 and 198 mg., and chlorides, from 730 to 760 mg. The blood showed a high grade of secondary anemia and eosinophilia. A heavy infection with *Strongyloides* was present. Nephrosis with a large white kidney was suspected, but operation for bilateral decapsulation revealed small red granular organs. The therapeutic response to diets poor in salt was fairly good, but the response to diuretics, especially methaphen, was spectacular. The patient would leave the hospital only to return with a recurrence. The patient died in uremia.

The diagnosis was chronic nephritis with edema uremia and secondary damage to the heart and vessels.

Case 5 (numbers 7 and 14 in table 1).—A. H. P., a white night watchman, aged 65, suffered from paroxysmal attacks of severe nocturnal dyspnea, cardiac asthma, some dyspnea on exertion, pain in the left side of the chest on deep inspiration, weakness, edema of the dependent parts and hemoptysis. The milder symptoms of cardiac failure had been present for two years, but the grave manifestations had been present for only about four months. The patient was obese and hypersthenic, with conspicuous cyanosis of the lips and mucous membranes, telangiectasis, xanthelasma, engorgement of the veins of the neck, enlargement of the heart, an apical systolic murmur, occasional premature contractions, sinus tachycardia, practically unchanged peripheral blood vessels and a blood pressure of 130 systolic and 100 diastolic. Râles were heard at the bases of the lungs; the liver was enlarged, and edema was present in the dependent parts.

The urine varied in specific gravity from 1.015 to 1.022 and contained a trace of albumin and hyaline casts. The excretion of phenolsulphonphthalein was 40 per cent in two hours. The blood chemistry was reported normal; nonprotein nitrogen, 33 mg.; uric acid, 3 mg.; blood sugar, 152 mg. Roentgenograms taken at a distance of 2 meters showed a cardiac shadow measuring 19.2 cm. across and an aortic hemicircle of 5.1 cm. The electrocardiograms showed minute complexes and right and left ventricular ectopic complexes.

The diagnosis was heart failure with edema and congestion of the lungs, liver and kidneys, myocardial insufficiency from a probable adhesive pericarditis.

The patient died, but permission for autopsy was refused.

Case 6 (number 6 in table 1).—G. J., a white lumberjack, aged 38, was troubled with vertigo, headaches, pain in the right flank, bloody urine and occasionally sudden attacks of unconsciousness and blindness, weakness and palpitation. His symptoms had been intermittently present for about eighteen months and had gradually increased in severity and in frequency. Overexertion seemed to be a precipitating factor.

The patient presented a robust appearance. A chronic suppurative otitis media and a chronic sinusitis were present. The heart was enlarged, and the retromanubrial dulness was increased. The aortic second sound was loudly accentuated. Significant murmurs were not heard. The brachial and radial arteries were slightly, if at all, sclerosed. The blood pressure was as high as 220 systolic and 106 diastolic and averaged 190 systolic and 80 diastolic. The liver was just palpable and was slightly tender, and the kidneys, especially the right, were sensitive to pressure.

The urine showed a specific gravity of 1.016 and contained a heavy trace of albumin, blood and granular and hyaline casts. Ureteral catheterization revealed the right kidney to be the source of the blood. The excretion of phenolsulphonphthalein in two hours was 95 per cent at one test and 25 per cent at one test. The blood chemistry on two occasions was nonprotein nitrogen, 36 and 38; urea nitrogen, 18 and 19; uric acid, 3.8; calcium, 1.2 and 1.4 and blood sugar, 85 and 80 mg. The Wassermann reaction was negative. A secondary anemia was present. The patient improved. The diagnosis was subacute hemorrhagic nephritis.

Case 7 (number 33 in table 1).—J. H., a negro laborer, aged 66, entered the hospital because of a strangulated right inguinal hernia, and was operated on. Slight edema of the dependent parts, enlargement of the abdomen, oliguria and shortness of breath developed postoperatively, even though the patient remained in bed.

Icterus of the sclerae, moderate anasarca, râles at the bases of the lungs and ascites were the evidences of congestive heart failure with edema. The heart was enlarged; abnormal basal pulsation was noted and the retromanubrial dulness was increased. Sinus tachycardia and aortic diastolic and apical systolic murmurs were heard. The blood pressure was 220 systolic and 100 diastolic.

The urine showed a specific gravity of 1.020 and contained a trace of albumin with many granular and hyaline casts. The phenolsulphonphthalein excretion was 50 per cent at one test and 25 per cent once in the two hour period. The blood chemical analysis showed: nonprotein nitrogen, 60 and 40; calcium, 1.3 and 1.1; chlorides, 640 and 730 mg., and carbon dioxide, 66 volumes per cent. The Wassermann reaction was negative on two occasions. The blood showed a secondary anemia. The ascitic fluid that was frequently removed had the characteristics of a transudate. The electrocardiograms showed a great left ventricular predominance.

The diagnosis was congestive heart failure with edema and ascites and syphilitic aortic regurgitation.

Case 8 (number 16 in table 1).—N. B., a Scotch physician and soldier of fortune, aged 70, began to have symptoms of cardiac failure following influenzal pneumonia. In spite of pollakiuria, he began to have swelling of the extremities and abdomen accompanied by dyspnea and nausea. The symptoms continued and increased in spite of adequate therapy.

Besides the symptoms described, a generalized anasarca and a distended abdomen, moderate cyanosis and orthopnea, congestion of the veins of the neck, enlargement of the heart, accentuation of the aortic second sound, no significant murmur, râles at the bases of the lungs, an enlarged and tender liver and ascites were present. The peripheral vessels apparently were not abnormal. The blood pressure was 190 systolic and 122 diastolic.

The urine showed a specific gravity of 1.018 and contained 2 per cent moist albumin, hyaline and fine granular casts, many pus cells and red blood cells. The test for diuresis showed the ability of the kidney to concentrate 1.022, but an inability to secrete much urine. The excretion of phenolsulphonphthalein was 15 per cent. The blood chemistry was reported as follows: nonprotein nitrogen, 54; uric acid, 4; calcium, 1.5. The icterus index was 10. The direct and indirect van den Bergh tests were positive. Polymorphonuclear leukocytosis was present. The roentgenogram showed an aortic hemicircle of 5.7 cm. and a transverse heart measurement of 17.5 cm. The electrocardiograms showed a left ventricular predominance.

The diagnosis was cardiac failure, chronic myocarditis, chronic nephritis and hypertension.

CASE 9 (number 13 in table 1).—A. G., an Acadian farmer, aged 45, came to the hospital because of a chronic cough with expectoration and asthmatic wheezing. His trouble had begun with attacks of bronchitis during the previous five winters. The attacks had become more and more persistent, and shortness of breath and edema of the extremities appeared.

The patient presented the signs of a chronic asthmatic bronchitis, emphysema and sibilant and sonorous râles. The heart was apparently not enlarged; the sounds were distant; the blood pressure was 130 systolic and 90 diastolic; the peripheral vessels were slightly sclerosed. The liver was just palpable and was slightly tender. Slight edema was present in the pretibial tissues.

The specific gravity of the urine was 1.020; it contained a slight trace of albumin and a few hyaline and granular casts. The excretion of phenolsulphonphthalein was 45 per cent in two hours. The blood chemistry revealed a nonprotein nitrogen of 40 mg.

The diagnosis was asthmatic bronchitis, emphysema and chronic nephritis.

CASE 10 (number 23 in table 1).—E. J., a negress, aged 23, in the sixth month of her first pregnancy, began to vomit, suffer headaches and have convulsions. She had not had any previous symptoms—no urinary disturbance or edema and no known serious infectious disease that might have been an etiologic factor for renal damage.

The eyes were prominent but otherwise not abnormal. Foci of infection were not in evidence in the nose and throat. The heart was not definitely enlarged, and nothing significant was observed. The blood pressure ranged from 210 systolic and 140 diastolic down to 190 systolic and 120 diastolic. Dulness in the liver area was diminished, and the uterus was enlarged to the size for a six months' pregnancy. Edema was not present.

The urine varied in specific gravity from 1.005 to 1.030 and contained from 10 to 20 per cent moist albumin, red blood cells and hyaline casts. The excretion of phenolsulphonphthalein was 50 per cent in two hours. The blood chemical analysis revealed: nonprotein nitrogen, 30; uric acid, 4; calcium, 1.2; chloride, 409 mg. per hundred cubic centimeters. The Wassermann reaction was negative. Anemia was not present. Electrocardiograms showed sinus tachycardia but did not show predominance. Venesection, with the repeated withdrawal of 500 cc. of blood, and even the discharge of the fetus did not produce any significant change in the hypertension.

The diagnosis was eclampsia, toxemia of pregnancy, acute hemorrhagic nephritis with gross hematuria and hypertension.

CASE 11 (number 31 in table 1).—W. M., an American storekeeper, aged 37, had been troubled for a year with pains in the head, the right flank, the perineum and the genitals, with hematuria. The throbbing occipital headache at times became almost unbearable; at such times with vertigo spots appeared before the eyes and the hematuria became most severe. Intermittence was noticed in the severity of symptoms. Some dyspnea on exertion or excitement and nocturia had been noted. The symptoms were aggravated by food. The patient had had four attacks of renal colic in which gravel had been present in the urine from eight to six years before the present illness. Gonorrhea when he was 20, pneumonia in the same year, a questionable lesion on the penis when he was 30 and four stillbirths in the family were considered significant points in the history.

The face was flushed and the eyelids were puffy. The temporal arteries were tortuous and the brachial and radial arteries slightly thickened. The blood pres-

sure was 290 systolic and 190 diastolic; it ranged as low as 220 systolic and 140 diastolic. The heart was slightly enlarged, the apex extending as much as 2 cm. outside the midclavicular line; the aortic second sound was ringing, but no murmurs were heard.

The urine showed a specific gravity of 1.018 and contained 1 per cent moist albumin, red blood cells and occasional pus cells. The excretion of phenolsulphonphthalein was 50 per cent in two hours. The blood nonprotein nitrogen was 32 mg. and the chlorides, 472 mg. Anemia was not present. The Wassermann reaction was negative.

The diagnosis was hyperpiesia, nephritis (?) and early arteriosclerosis.

CASE 12 (number 22 in table 1).—W. E. G., a white stationary engineer, aged 58, had had headaches, vertigo, positive scotomas, cough, dyspnea, weakness and pollakiuria. The symptoms had caused him to change his occupation to that of night watchman two years before admission. He had known that he had high blood pressure at the age of 48. He had had two attacks of pneumonia and typhoid fever and gonorrhea.

He presented slight pallor, puffiness about the eyes, conspicuous sclerosis of the temporal, brachial, radial and retinal arteries, enlargement of the heart, accentuation of the aortic second sound and a blood pressure of 240 systolic and 135 diastolic.

The specific gravity of the urine varied from 1.010 to 1.015; the urine contained a slight trace of albumin and hyaline and granular casts with occasional red blood cells. The concentration test for diuresis indicated an inability to secrete urine. The excretion of phenolsulphonphthalein was 30 per cent; the nonprotein nitrogen, 45; uric acid, 2; calcium, 1.3 and blood sugar, 95 mg. The Wassermann reaction was negative. Significant anemia was not present. Electrocardiograms showed left ventricular predominance. The diagnosis was hyperpiesia, arteriosclerosis, chronic myocarditis and chronic nephritis.

CASE 13 (number 11 in table 1).—J. D., a white milk pedler, aged 31, presented himself at the clinic because of swelling of the abdomen and intermittent swelling of the legs, oliguria and diarrhea. He had had measles, mumps, whooping cough and gonorrhea, and had had a chronic addiction to alcohol.

The facies were typically hepatic with spider angiomas in the skin of the face and chest. Icterus of the sclerae, and crowned teeth which showed poor care, were present; the peripheral arteries were not sclerosed; the blood pressure was 110 systolic and 65 diastolic. The heart was displaced upward, with an accentuated pulmonic second sound. The lungs were slightly compressed; the abdomen was distended with ascitic transudate; the liver was enlarged, with superficial venous engorgement, but hemorrhoids were not present. Slight edema was found in the pretibial tissues.

The urine showed a specific gravity of 1.030 and contained urobilin and bile pigments. The stools contained bile. The blood serum contained some pigment. The ascitic transudate was removed, and on the administration of alkali, iodides and merbaphen, the patient improved remarkably.

The diagnosis was alcoholic and, questionably syphilitic, cirrhosis of the liver.

CASE 14 (number 24 in table 1).—S. W., a negress, aged 26, came to the hospital because of swelling of the abdomen and legs, a dry cough, dyspnea, vertigo, nausea, vomiting and nocturia. She was eight months pregnant and had had spontaneous miscarriages and one interrupted pregnancy.

The face was slightly puffy, and the expression was anxious. Some respiratory distress was evident. The heart was at the upper limit of normal in size, and an apical systolic sound, an accentuated pulmonic second sound and a blood pressure

of 204 systolic and 120 diastolic were noted. The abdomen was enlarged by the gravid uterus and a questionable ascites. The extremities were edematous.

The urine showed a specific gravity of 1.014 and contained 2 per cent albumin, hyaline casts and red blood cells. The concentration test for diuresis gave evidence of some difficulty in concentrating. The excretion of phenolsulphonphthalein was 45 per cent. The nonprotein nitrogen was 25 mg. and the chlorides, 528 mg. The electrocardiogram showed left ventricular predominance, sinus tachycardia, and changes in the T wave. The Wassermann reaction was variable.

Venesection was of temporary benefit. The delivery of twins gave some relief.

The diagnosis was eclampsia (nephritic) toxemia of pregnancy and myocardial insufficiency (?).

Case 15 (number 26 in table 1).—C. J., a negro laborer, aged about 60, was admitted to the hospital because of inability to walk. For a year he had been completely disabled with pains in the muscle joints and the girdle region, vertigo, headaches, forgetfulness, difficulty in articulation, incontinence of urine, tremors of the hands and feet, inability to walk, because of falling forward when attempting to do so, and dyspnea and swelling of the feet and legs at times. He had not had venereal disease.

He presented a shuffling gait, staccato speech, fine tremor of the lips, tongue, hands and feet, diminution of the knee reflexes, fixation of the pupils, arcus seniles and well defined peripheral arteriosclerosis. The blood pressure was 150 systolic and 100 diastolic; the heart was enlarged to the left and downward. Slight edema was present in the pretibial tissues.

The urine showed a specific gravity of 1.012 and contained a trace of albumin and a few hyaline casts. The excretion of phenolsulphonphthalein was 40 per cent in two hours. The blood chemistry showed a nonprotein nitrogen value of 37 mg. The Wassermann reaction was negative. Electrocardiograms showed left ventricular preponderance and frequent auricular right and left ventricular ectopic beats and slight tachycardia.

The patient was given six doses of 0.6 Gm. of neoarsphenamine followed by a course of mixed treatment. He was discharged with the diagnosis of general arteriosclerosis, chronic myocarditis, arteriosclerotic or syphilitic taboparesis (?).

Case 16 (number 21 in table 1).—J. B., a white upholsterer, aged 62, suffered from cough and paroxysmal dyspnea at night, which began suddenly six months before examination when he was awakened with the sensation of drowning. The administration of epinephrine relieved him of the attack, but weakness, palpitation and dyspnea on slight exertion became noticeable. Cough, hemoptysis, epistaxis, nocturia, puffiness about the eyes and severe attacks of paroxysmal dyspnea recurred in a month at intervals of three and two weeks and then practically nightly.

Malaria, typhoid fever, dysentery, gonorrhea, buboes and syphilitic infection at 21 years and chronic alcoholism were etiologic factors.

The patient was small and emaciated and presented prominent and tortuous temporal, brachial and radial arteries and pulmonary emphysema with asthmatic bronchitis. Enlargement of the heart, accentuation of the aortic second sound and blood pressures of 210 systolic and 125 diastolic and 180 systolic and 105 diastolic were present.

The urine showed a low specific gravity of 1.010 and contained 4 per cent albumin and many hyaline and coarsely granular casts. The excretion of phenolsulphonphthalein was 50 per cent. The blood chemistry was: nonprotein nitrogen, 34; urea nitrogen, 17; uric acid, 3.1; calcium, 1.3 and blood sugar, 95 mg. Electro-

cardiograms showed left ventricular preponderance and suggestive evidence of defective intraventricular conduction.

The diagnosis was hypertension, advanced arteriosclerosis, chronic nephritis, chronic myocarditis, asthmatic bronchitis, pulmonary emphysema and edema.

CASE 17 (number 29 in table 1).—W. R., a negro laborer, aged 38, with a history of syphilitic infection, presented symptoms and signs of vascular involvement of the central nervous system, vertigo, headaches, disturbance of speech, weaknss in the left arm and both legs with palpitation, enlarged heart, an apical systolic murmur, a ringing aortic second sound, tortuous and thickened brachial and radial arteries and a blood pressure of 260 systolic and 160 diastolic.

The urine showed a specific gravity of 1.020 and contained on one occasion a trace of sugar and at other times a trace of albumin and a few pus cells. The excretion of phenolsulphonphthalein was 60 per cent in two hours. The blood chemical analysis showed: nonprotein nitrogen, 34 mg.; uric acid, 4 mg.; calcium, 1.3 mg. and blood sugar, 90 mg. The Wassermann reaction in the blood was strongly positive. The spinal fluid was negative. The roentgenogram taken at a distance of 2 meters showed the transverse diameter of the heart to be 16.1 cm. and that of the aortic hemicircle to be 5.2 cm. Electrocardiograms gave evidence of left ventricular predominance. Ophthalmoscopic examination revealed silver wire arteries and dilated veins in the fundus.

The diagnosis was hyperpiesia, arteriosclerosis, especially of the cerebral vessels, and relatively little renal impairment.

CASE 18 (number 32 in table 1).—F. W., a white farmer, aged 47, noted numbness of the feet for a year, then nocturnal dyspnea, occipital headaches, increasing listlessness and weakness, palpitation and intermittence of heart action and loss of weight.

The patient presented generalized lymphadenitis. The pupillary reactions to light were sluggish. The chest was rachitic; the heart moderately enlarged; the aortic second sound was accentuated and slightly ringing; the brachial and radial arteries were thickened and the retinal arteries small. The blood pressure was 210 systolic and 165 diastolic and 180 systolic and 100 diastolic. Slight edema of the ankles was noted.

The specific gravity of the urine varied in the concentration test between 1.008 and 1.017, and a heavy trace of albumin and finely granular and hyaline casts were found. The excretion of phenolsulphonphthalein was 60 per cent. The blood chemistry studies revealed: nonprotein nitrogen, 60; uric acid, 3.5; calcium, 1.6; blood sugar, 100 and chlorides, 492 mg. The Wassermann reaction was negative. A slight secondary anemia was present. The electrocardiograms showed small complexes and a slight left ventricular predominance.

The diagnosis was hyperpiesia, arteriosclerosis and chronic nephritis.

CASE 19 (number 12 in table 1).—W. M., a white pipefitter, aged 33, was admitted to the hospital because of intense persistent jaundice, pruritus and weakness. Four years previously he had been operated on for appendicitis, a year later for adhesions and in another year for gallstones. Four days after the last operation, jaundice appeared and persisted. This was one year previous to admission. A few months later, he became jaundiced. He bled so severely following the extraction of a tooth that transfusion of blood was necessary. The jaundice apparently disappeared spontaneously for a few months after this, only to reappear at the sixth month, associated with enlargement of the liver, pruritus, blurring of vision and loss of weight.

He presented deep jaundice of the sclerae, mucous membranes and skin. The heart was not enlarged, and the vessel walls were soft. The heart rate was 86 a

minute, and the blood pressure was 108 systolic and 70 diastolic. Some abdominal tenderness, an enlarged, smooth liver and an irregular fever were noted.

The specific gravity of the urine was 1.025 and bile, a trace of albumin and epithelial and occasional pus cells were noted. The excretion of phenolsulphonphthalein was 65 per cent in two hours. The blood chemical analyses showed: nonprotein nitrogen, 70; calcium, 1.2; chlorides, 560; blood sugar 90 mg., and carbon dioxide, 74 volume per cent. The Wassermann reaction was negative; the icterus and index was 100 times that of the normal; a slight leukocytosis of 13,500 was present, and bile was absent from the stool.

The diagnosis was complete obstructive jaundice of mechanical origin.

CASE 20 (number 35 in table 1).—F. B., a white carpenter, aged 75, was admitted to the hospital for the last time in his fifth attack of generalized edema. He had had dyspnea and cardiac weakness for two and a half years, with congestive failure at intervals and especially after any undue exertion.

Besides general anasarca, he presented orthopnea, cyanosis, engorged veins in the neck, emphysema, great cardiac enlargement, blurting prolongation of the mitral first sound and a distinctly accentuated aortic second sound. The blood pressure was 185 systolic and 135 diastolic; the lungs were emphysematous and moist; the abdomen was tense with ascites; the liver was enlarged, and the extremities were dropsical.

The urine showed a specific gravity of 1.012 and contained a slight trace of albumin with many hyaline and granular casts. The excretion of phenolsulphonphthalein was 17 per cent in two hours. The nonprotein nitrogen was 50; uric acid, 4; calcium, 1.8 and blood sugar, 90 mg. The electrocardiogram showed complete block of the right bundle branch. The patient died.

The diagnosis was congestive heart failure, chronic myocarditis, chronic nephritis and hypertension.

CASE 21 (number 25 in table 1).—G. L., a bartender, aged 59, was brought into the hospital practically comatose with general anasarca. He had been troubled with dyspnea, palpitation and tachycardia, abdominal swelling and oliguria. He had been a chronic drinker of beer.

Besides generalized edema, he presented orthopnea, cyanosis, engorgement of the veins in the neck, an enlarged heart, a systolic murmur, a rapid regular heart rhythm that could be slowed by pressure on the vagus, slight peripheral arteriosclerosis, a blood pressure of 160 systolic and 110 diastolic, questionable enlargement of the liver and ascites.

The urine showed a specific gravity of 1.022 and contained a trace of albumin, a few hyaline casts and red blood cells. The excretion of phenolsulphonphthalein was 25 per cent in two hours. The blood chemistry showed: nonprotein nitrogen, 78 and chlorides, 450 mg. The Wasserman reaction was negative. Electrocardiograms showed auricular flutter and, after digitalization, impure flutter and finally fibrillation, which persisted. The patient died of extreme congestive cardiac failure.

The diagnosis was congestive heart failure with general anasarca, chronic myocarditis, chronic nephritis, hypertension, auricular flutter and fibrillation.

CASE 22 (number 27 in table 1).—C. W., a negro laborer, aged 42, was admitted because of swelling of the body, headache, some cough and dyspnea. He had had previous attacks of edema, but none as severe as the one which had brought him to the hospital and which had been increasing slowly in severity for several weeks. He had had gonorrhea and a probable syphilitic infection when he was younger.

Besides puffiness of the face, he presented moderate edema of the lower extremities, enlargement of the heart, accentuation of the aortic second sound and a slight aortic systolic murmur, slight sclerosis of the peripheral arteries and a blood pressure of 162 systolic and 92 diastolic.

The urine showed a specific gravity of 1.012 and contained 2 per cent albumin, many hyaline and granular casts and an occasional red blood cell. The blood chemical studies revealed nonprotein nitrogen, 82 and chlorides 410 mg. The Wassermann reaction was positive. A mild secondary anemia was present.

The diagnosis was syphilitic aortitis, chronic myocarditis and chronic nephritis with hypertension and edema.

CASE 23 (number T 5 in table 1).—J. K., a white cook on a ship, aged 50, entered the clinic because of edema of the legs and genitals. Previous but less extensive dropsical states had been experienced during the preceding two years, together with increasing dyspnea, orthopnea, weakness, headache, vertigo and persistent cough. A syphilitic infection when he was 30, although he had been treated by injections for seven months, must have been an etiologic factor.

He presented pallor, pastiness, puffy eyelids, cardiac enlargement, accentuated aortic second sound, a pericardial rub, occasional ectopic heart beats, a blood pressure of 225 systolic, 120 diastolic, edema of the lungs, hydrothorax, ascites and anasarca.

The urine varied in specific gravity from 1.010 to 1.020 and in albumin content from 16 to 40 per cent. A few hyaline and coarsely granular casts were present. The excretions of phenolsulphonphthalein was, respectively, 15 per cent, 20 per cent and 40 per cent in two hour periods. Blood chemical studies revealed: nonprotein nitrogen, 62 and 50; urea nitrogen, 29; calcium, 2.5; blood sugar, 95 and chlorides, 644. High grade secondary anemia and leukocytosis were present. The electrocardiograms showed left ventricular predominance and changes in the T wave.

At autopsy, the kidneys weighed 240 Gm. each and showed a narrow cortex, injected blood vessels, small cysts, increased connective tissue and casts in the tubules.

The diagnosis was acute exacerbation of a chronic nephritis, hypertension and chronic myocarditis with congestive heart failure.

CASE 24 (number 36 in table 1).—E. B., a white telephone operator, aged 44, had fallen unconscious in his quarters for the third time. He had previously been admitted to the clinic in coma, with paresis and pain in the left arm and leg. Severe headache, dizziness, weakness and numbness and short periods of unconsciousness had been troublesome for years. Dyspnea and precordial pain had been noted for a year. He had had acute nephritis followng scarlet fever at the age of 8 and had had hypertension ever since. His father, mother and six sisters had died of disease of the kidneys.

The patient presented nephritic facies with a definite exophthalmos and dilated venules of the skin. Other symptoms included carious teeth; cardiac enlargement; accentuated aortic second sounds; slight arteriosclerosis; a labile hypertension ranging from 270 systolic and 160 diastolic down to 165 systolic and 105 diastolic; emphysematous lungs; amputation of the left foot and slight edema of the extremities.

The urine showed a specific gravity of 1.012 and contained 12 per cent moist albumin and many hyaline and granular casts. The excretion of phenolsulphonphthalein was 5 per cent and 0 per cent in two hours. The blood chemistry studies

revealed nonprotein nitrogen, 95; urea nitrogen, 45; calcium, 4.4. The Wassermann reaction was negative. The patient improved on the administration of benzyl succinate.

The diagnosis was chronic nephritis, hypertension and cerebral angiospasm.

CASE 25 (number 2 in table 1).—B. S., an Acadian fisherman, aged 65, came to the clinic at Charity Hospital because of chronic persistent headache, dizziness, weak spells, shortness of breath and swelling of the feet and legs. He had had these symptoms for a year, but there had been a definite increase in the severity of his troubles. Palpitation and dyspnea were noted on slight exertion, and occasionally he had paroxysmal attacks of smothering asthma. He frequently suffered from nausea, but never vomited. He was chronically constipated. Urinary frequency every half hour or every hour night and day troubled him considerably. His light-headed sensation was extreme at times, and he "fell out" but never lost consciousness. The administration of glyceryl trinitrate and amyl nitrite relieved his headache.

He presented the usual signs of senility, but evidences of discomfort were not seen. Arcus senilis was present in each eye. The mouth contained a few remaining carious snags, but not any sound teeth. The cardiac dulness was definitely increased. The aortic and mitral second sounds were loudly accentuated. A blowing systolic murmur was heard, and an occasional premature contraction disturbed the rhythm. The peripheral blood vessels were definitely sclerosed. The blood pressure was 280 systolic, 140 diastolic, but dropped to 260 systolic, 125 diastolic and later to 230 systolic and 120 diastolic, with the administration of apothesine, intrathecally, the drop in blood pressure was from 250 systolic, 140 diastolic to 201 systolic, 110 diastolic in twenty-five minutes. The liver was enlarged but not tender. Slight edema was present in the pretibial tissues.

The urine was clear and acid in reaction; the specific gravity was 1.012; it did not contain albumin or casts. The excretion of phenolsulphonphthalein was 20 per cent in two hours. The chemical analyses of the blood revealed: nonprotein nitrogen, 48 mg. per hundred cubic centimeters; urea nitrogen, 12; uric acid, 4.0 and calcium, 1.2. The Wassermann reactions with the blood and spinal fluid were negative. Electrocardiograms showed left ventricular predominance with junctional and auricular ectopic beats.

The diagnosis was hyperpiesia, arteriosclerosis, chronic myocarditis and chronic nephritis.

CASE 26 (number 30 in table 1)).—A. S., an American clerk, unmarried, aged 62, came to the hospital because of vomiting and faintness. Definite symptoms had not been present until the day before admission, when an attack of nausea, dizziness, faintness and syncope occurred. He had had gradual failing of vision and spots before his eyes and severe headaches every morning associated with nausea and occasional vomiting. One year before admission, while working, he had a severe attack of vertigo which was followed by a free epistaxis which continued for about eight hours. He was relieved of the symptoms for several weeks. Within the year before admission, he had had eight or ten attacks of palpitation which came on suddenly and lasted five or ten minutes but did not reappear again for about a month. He had had frequency of micturition for many years. He had had typhoid fever at 10 years of age, acute rheumatic fever at 12, pneumonia at 15 and influenza in 1918.

The patient was decrepit. His heart was somewhat enlarged. The aortic second sound was loud and metallic. The sounds at the apex were short and slightly valvular. Blood pressure had been as high as 250 systolic, 155 diastolic

and as low as 200 systolic, 120 diastolic. The lungs were emphysematous. The abdomen showed conspicuous abdominal breathing and scars in each groin. The radial and brachial arteries were increased in size and were tortuous and thickened. Some edema was present in the pretibial tissues.

The urine showed a specific gravity of 1.010 and contained 1 per cent albumin and many hyaline and granular casts. Blood chemical determinations showed nonprotein nitrogen, 84; urea nitrogen, 50; uric acid, 4; calcium, 3; blood sugar, 90 mg. The blood Wassermann reaction was negative. The blood picture showed a slight secondary anemia.

The diagnosis was hyperpiesia, arteriosclerosis, chronic myocarditis and chronic nephritis.

CASE 27 (number 3 in table 1)).—W. H., a white unmarried millwright, aged 59, came to the hospital because of swelling of the feet, ankles and abdomen and shortness of breath. His first symptoms, slight swelling of the abdomen and shortness of breath, were noted seven months previously. After his abdomen began to swell, he noted smothering spells on straining at stool and urination. The edema, especially the puffiness about the eyelids and the swelling of the genitals and even some of the waterlogging of the legs would at first disappear on rest in bed, but gradually the symptoms would not respond to the rest treatment. At times, the dyspnea was so severe that the patient was orthopneic. He had had a sore, which was presumably a syphilitic lesion, at the age of 24. At the age of 48, he took four injections of arsphenamine. He had had frequency of urination, urinating from eight to ten times a day and from five to six times at night. The urine had been smoky at times.

Physical examination revealed a more or less general anasarca, with puffiness of the face as well as the dependent parts. The heart was slightly overactive and distinctly enlarged. A faint systolic murmur was heard at the apex. The aortic second sound was accentuated. The blood pressure which was 180 systolic and 135 diastolic on admission dropped to 165 systolic and 105 diastolic. The rhythm of the heart was made irregular at times by premature contractions. The lungs were emphysematous. The abdomen was enlarged. The fluid waves were evident. The liver was not palpable. The spleen was palpable on deep inspiration.

The urine contained 2 per cent albumin, hyaline and fine and coarse granular casts and a few pus cells. The specific gravity was 1.010 and 1.014. The excretion of phenolsulphonphthalein was 5 per cent in two hours. The blood showed a secondary anemia; a definite retention of nitrogen; nonprotein nitrogen, 90; urea nitrogen, 55; uric acid, 56; calcium, 3.2 mg. The blood Wassermann reaction was negative. The electrocardiogram showed negative T waves in lead I and left ventricular predominance. The patient was given cathartics, and abdominal paracentesis was done with the removal of about 10 liters of fluid on Oct. 20, 1926. On October 25, the edema was subsiding. On October 27, practically all of the edema had disappeared. The patient continued on a salt-free diet, but dyspnea on exertion continued.

The diagnosis was chronic nephritis and hypertension and chronic myocarditis with congestive failure.

CASE 28 (number 28 in table 1).—F. P., a negro farmer, aged 24, was brought to the hospital complaining of swelling of the feet and legs which had been present for ten weeks and which had occurred in two previous attacks in the two preceding winters. Besides edema of the extremities, abdomen and face, he had had dull headache, positive scotomas, abdominal pain and oliguria. In the past he had had pneumonia, gonorrhea and syphilis. For three generations, a total of seven of his relatives had died in a dropsical condition.

He presented generalized anasarca, with puffiness of the face and distention of the abdomen. The retinal arteries were markedly tortuous with widened arterial bands. The peripheral vessels were only slightly thickened. The blood pressure was 185 systolic and 134 diastolic; the heart was enlarged, with an accentuated second sound, but without significant murmurs. Striae were conspicuous over the abdomen; ascites, an enlarged liver, and tenderness were also found.

The specific gravity of the urine varied between 1.005 and 1.010; it contained from 20 to 23 per cent moist albumin, hyaline and granular casts; pus and red blood cells were found. The excretion of phenolsulphonphthalein was 5 and 8 per cent. There were limitations of the concentration test for diuresis in both processes. Blood chemical studies showed: nonprotein nitrogen, 99 and 125; uric acid, 4; calcium, 1.8 and blood sugar, 100 and 95 mg. per 100 cc.

The diagnosis was chronic nephritis with edema and hypertension.

At autopsy, the kidneys were pale pink and firm with increased connective tissue and contraction of the cortex. The capsules stripped with difficulty, leaving an irregularly granular surface.

Case 29 (number 7 in table).—E. S., an Italian spinner, aged 20, came to the hospital because of weakness, drowsiness and shortness of breath. He had not worked for several years. Three years before he came to the hospital, his left kidney had been removed for abscess. He had also had his tonsils removed, but he never did recover. In August, 1926, he began to feel weak, and his feet and ankles swelled moderately. Shortness of breath suddenly developed and was noted even when he was quietly walking about. He continued to receive some hypodermic injections from his family physician, but without any improvement. Severe headache, worse at night, developed and remained more or less constant in the frontal region. He had dyspnea, tinnitus and was more or less deaf; once or twice a week, he would have copious epistaxis. He began to lose his appetite and he vomited on three or four occasions. His urine was dark and smoky, and he had a definite fever. He attributed this to a respiratory infection that he had had for about three weeks. He was rarely bothered by nocturia. The patient had had typhoid fever for two months at the age of 11 and influenza in 1918. In 1923, he began to have epistaxis. He felt a bit weak after control of the hemorrhage but did not have other symptoms. In May, 1924, he noticed blood in the urine and pain in the region of the left kidney. The kidney was removed. A month later, he had his tonsils removed because of a possibility of their being a focus of the kidney trouble.

When seen, the patient was propped up in bed and was somewhat drowsy and listless. The skin was pale, sallow and of a somewhat waxy yellowish tint. There was some puffiness over the eyelids. He had a slight exophthalmos and a subconjunctival hemorrhage. His hearing was definitely impaired. The mucous membrane and lips were pale. The heart impulse was well located in the midclavicular line in the fifth interspace. Sounds were distinct. Rhythm was regular, the rate being 86 per minute. Murmurs were not heard. The pulmonary second sound was accentuated. The blood pressure was 160 systolic and 90 diastolic. The lungs were normal. The abdomen showed nothing of significance except the scar in the region of the left kidney. The liver and spleen were not palpable. Some edema of the extremities was present.

The urine showed a specific gravity of 1.010 and contained 3 per cent albumin and occasional finely granular casts. The excretion of phenolsulphonphthalein was 0 per cent in two hours. The blood showed a high secondary anemia and slight leukocytosis. The Wassermann reaction was negative. Blood chemical studies

showed: nonprotein nitrogen, 100; urea nitrogen, 49; uric acid, 46; calcium, 3.9; blood sugar, 75 mg. per hundred cubic centimeters. The patient's uremic coma increased. He had one convulsion on Nov. 3, 1926, that lasted five minutes. The pulse rate increased to 108. The temperature fluctuated from subnormal to 99.6. Other phenolsulphonphthalein tests showed complete retention of the dye for two hours. Blood pressure rose to 225 systolic and 80 diastolic, and venesection was done. One hundred and fifty cubic centimeters of blood was removed. Glucose solution was given intravenously but with no avail. The patient died, Nov. 4, 1926. Autopsy was refused.

The diagnosis was uremia, chronic nephritis with subacute exacerbation, hypertension and severe secondary anemia.

CASE 30 (BB in table 1).—W. L., a negro section hand, aged 39, came to the clinic complaining of swelling of the legs and abdomen and pain over the heart. His illness dated back only one week, according to his story, although he had had symptoms of cardiac failure for at least six months. He had noted a swelling of the feet after getting wet one week previously; the swelling gradually increased and progressed upward until the abdomen was involved. Shortness of breath, attacks of palpitation, dizziness and cough, with the expectoration of bright red blood, had been symptoms of increasing severity. He had had to get up four and five times each night to urinate. Nausea and some constipation had been present. He had had gonorrhea and inflammatory rheumatism when he was 20, a chancre at the age of 24 and had been treated with intravenous injections of what most likely was arsphenamine. He had also been chronically addicted to alcohol.

The physical examination revealed a general anasarca, a moderately hard edema of the lower extremities and genitals, sluggish pupils and diminished reflexes. The heart was definitely enlarged. A diastolic murmur replaced the aortic second sound and was transmitted to the left border of the sternum, but changed at the apex from its high pitch to a low, rumbling character. The blood pressure was 126 systolic, 38 diastolic. The liver was engorged.

The urine was acid, with a specific gravity of 1.024, a slight trace of albumin and a few red cells, pus cells and granular casts in the sediment. The excretion of phenolsulphonphthalein was 30 per cent.

The diagnosis was chronic nephritis, chronic syphilitic aortic disease with myocardial insufficiency. Autopsy revealed a chronic nephritis and chronic aortic valvulitis.

Autopsy revealed a chronic nephritis and chronic aortic valvulitis.

CASE 31 (number 34 in table 1).—M. P., a colored teamster, aged 32, had suffered for four months with shortness of breath on exertion, epistaxis, irregular, painful vomiting of cloudy fluid, nocturnal vertigo and pollakiuria.

He had had malaria at the age of 10, gonorrhea twice and a chancre at 22, for which he did not receive treatment.

Physical examination at the time of admission revealed that the patient was drowsy and dyspneic, with moderate edema of the ankles and feet and at the bases of both lungs. The inguinal glands were palpable. Retrosternal dulness was increased. The heart was enlarged to the left and downward. A blowing systolic murmur was heard at the apex. The aortic second sound was accentuated. The blood pressure was 190 systolic, 108 diastolic. The liver was felt 6 cm. below the costal margin.

The blood Wassermann reaction was strongly positive. The urine showed a specific gravity of from 1.005 to 1.010. Albumin was constantly present in small quantities, and hyaline casts were found on each examination. The blood picture was that of a moderate secondary anemia. The excretion of phenolsulphonphthal-

ein was 3 per cent. Blood chemical studies showed: nonprotein nitrogen, 300; calcium, 7; uric acid, 6.6; blood sugar, 85 mg.

The diagnosis was chronic nephritis, hypertension, chronic syphilitic aortitis and myocarditis with congestive failure.

The autopsy revealed small contracted granular kidneys with numerous cystic formations, hypertrophy and dilatation of the heart, aortitis and edema of the lungs.

SUMMARY

Analyses were made to determine the distribution of the nonprotein sulphur fractions of the blood in whole blood and in plasma obtained from a series of cases representing various pathologic conditions of the kidneys, heart and liver.

The results obtained would seem to indicate that while in normal persons the inorganic sulphate fraction is present only in plasma, in patients with nephritis who show retention of sulphate the inorganic sulphate content of the whole blood rises more rapidly than does the concentration of this fraction in the plasma, indicating diffusion and storage of this constituent in the corpuscles.

There does not appear to be any direct relation between the retention of nonprotein nitrogen and inorganic sulphate, or between the latter, the neutral sulphur fraction or the chlorides.

THE CARDIOTACHOMETER

AN INSTRUMENT TO COUNT THE TOTALITY OF HEART BEATS OVER LONG PERIODS OF TIME *

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From the earliest days of medicine the study of the pulse has been of major interest to physicians, and trained observation of the pulse at the bedside has been of fundamental importance in diagnosis and in treatment. The quality of the pulse gives information about the condition of the heart and arteries, and, in former years, was supposed to reflect specific abnormal bodily states. Up to 100 years ago, the practicing physician paid little attention to pulse rate, but indulged in hair-splitting refinements of classification of pulses of different qualities. Innumerable voluminous but sterile treatises have been published on the quality of the pulse. Quantitative studies of the pulse rate were initiated by Galileo (1620), who synchronized the beat of a pendulum with the pulse and expressed the pulse rate by the length of the pendulum. One hundred years later, Sir John Floyer published a book, called the "Physician's Pulse Watch," in which for the first time careful pulse-numerations are recorded. For this purpose, he employed a watch that ran sixty seconds. It was not, however, until the second quarter of the nineteenth century that counting the pulse was accepted as a routine procedure of importance.[1]

The pulse rate, which ordinarily coincides with the heart rate, is not alone a measure of the activity of the heart and so of one important component of its work, but it is also an indicator of the influence that many bodily functions and reactions exert on the heart. The introduction of the sphygmograph, the polygraph and the electrocardiograph have permitted a more intensive and accurate study of these changes in the rates of the heart and pulse, but the nature of these instruments has limited their usefulness in the investigation of this variation in rate. For the efficient operation of these instruments, the patient must be sitting quietly under more or less basal conditions. Recently, Goldschmidt [2] described an apparatus called the pulse resonator, which is designed to register instantaneous changes of rate over long periods of

* From the Medical Division of Montefiore Hospital for Chronic Diseases, New York.

1. Mitchell, S. W.: The Early History of Instrumental Precision in Medicine, Tr. Cong. Am. Phys. & Surg. **2**:179, 1892.

2. Kraus, F.; Goldschmidt, R., and Seelig, S.: Analyse des Pulsrhythmus mit dem Pulsresonator, Ztschr. f. d. ges. exper. Med. **53**:243, 1926.

time. The pulse resonator works on the principle of Galileo's pulsilogium. A series of pendulums of different periods are acted on by the pulsation of the radial artery, and the pendulum, the period of which corresponds to the pulse rate records electrically on a moving paper. This ingenious mechanism is a great improvement over previous ones for this purpose, but, as I shall point out in another communication, it is not altogether accurate. Furthermore, the patient must be at rest during the examination.

For some years I have been interested in devising an instrument that would register the totality of heart beats over a period of many hours, and that would function accurately while the patient was moving about actively. In the development of this instrument, I have been fortunate in having the cooperation of Dr. Benjamin Liebowitz, without whose technical knowledge and inventive skill the work would have been impossible.

We first attempted to operate a counter by means of an electrical contact, pneumatically actuated by the mechanical impulse of the heart beat or pulse beat. We found that this contact required a delicate adjustment, and that unless the patient remained quiet, too many extraneous factors were introduced. Muscular movement, coughing and jarring, for instance, would render the count inaccurate. We then attempted to pick up the heart beats microphonically, employing first a standard carbon granule microphone and later a telephone receiver the diaphragm of which made direct mechanical contact with the chest by means of a short wooden cylinder cemented to the diaphragm. Here, again, it became apparent that if the apparatus were made sufficiently delicate to register accurately every cardiac cycle, it would be necessary to keep the patient quiet, or the sound of his voice, a deep inspiration, or a jar of his body would introduce false registrations.

At the suggestion of Prof. H. B. Williams of Columbia University, we then undertook to test the feasibility of utilizing the action current of the heart. In our first attempts in this direction we led off the action current of the heart from the hands with the usual electrocardiographic electrodes and passed it through a three-tube, stock resistance coupled amplifier. It was possible to operate a sensitive relay and counter with this mechanism; but the apparatus was inadequate, allowing false registrations, as well as missing many heart beats. It became evident that the two chief requirements for success were a stable amplifier of large gain and more suitable electrodes. At this point, we were fortunate in enlisting the aid of Dr. Alfred N. Goldsmith, to whom we submitted the problem. He and his associate, Mr. Julius Weinberger, together with Messrs. Theodore A. Smith and George Rodwin, worked out the necessary theoretical and practical solution for the amplifier and for some of the accessory apparatus which is described below.

DESCRIPTION OF APPARATUS

The method employed consists in picking up the voltage of the action current of the heart with suitable electrodes, amplifying this voltage sufficiently, and passing the amplified current into a relay and counter system. The instrument devised to register the heart beats consists of the following elements: electrodes, amplifier, relay, counter and recorder.

The Electrodes.—Two shallow cup-shaped electrodes of monel metal 2.5 cm. in diameter and 0.5 cm. deep are employed (fig. 1). These electrodes are sewed to elastic tapes. The cup is filled with ordinary green soap, which serves as an electrolyte. When the electrodes are applied to the precordium, the tapes are tied around the chest and hold the electrodes in place. In most patients, the electrodes are best placed so that one is in the region of the apex beat and

Fig. 1.—The electrodes are sewed to elastic tape and tied around the chest.

the other in the second intercostal space to the right of the sternum. This is approximately the electrical axis of lead II of the electrocardiogram. Wires lead the current from the electrodes to the input terminals of the amplifier.

The Amplifier.—The wave form of the action current of the heart is complex, consisting usually of a main peak (the R wave of the electrocardiogram) and several smaller peaks and ripples. The main peak, which is used to operate the mechanism, is of well defined frequency, low in comparison with the acoustic frequencies handled ordinarily in the amplification of the speech; hence, a special type of amplifier is required. Furthermore, it would be desirable to amplify to a less degree the smaller peaks which are of lower frequency and the ripples which are generally of higher frequency. The amplification needed is high, probably of the order of 5,000 to 10,000 in voltage.

The straight resistance coupled amplifier, such as was used in the earlier experiments, has the disadvantage of instability at high gain; in addition, it is expensive and inconvenient. A combination of straight resistance and capacity

coupled circuits with inductance and capacity stages was tried out and was not found entirely satisfactory. When extreme amplification was used, this amplifier could not be made stable. Finally, a four-stage transformer coupled amplifier was designed, special precautions being taken; this arrangement was found stable, and the high amplification required was readily secured.

In order to pass the low frequencies, transformers of high primary impedance were used. To prevent oscillation, condensers of suitable size were placed across the primaries and secondaries of these transformers. These condensers cut the high frequency amplification down to practically zero, but do not influence the amplification of low frequencies to a great extent.

The wiring diagram of the amplifier is shown in figure 2. The overall amplification from input to the plate circuit of the last tube is about 20,000 in voltage at 35 cycles a second. A gain control of the shunt resistance type is placed across the input to the second stage to vary the overall amplification.

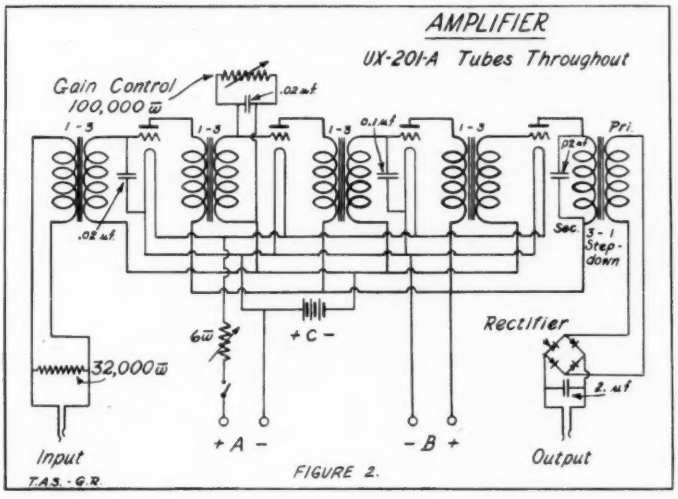

Fig. 2.—The wiring diagram of the amplifier.

Ordinary radio receiving tubes of the UX-201A type are employed with 90 volts B battery and 4½ volts negative C battery. The amplifier is mounted on a spring-suspended subpanel in a copper shielded box. The outside panel contains input and output jacks, starting switch and gain control. Leads coming through the back of the box connect to A and B batteries. The C battery is contained within the box.

The Relays.—In the original model a sensitive relay was made to operate by means of the plate current change in the final tube plate circuit. This system required that the constant final stage plate current be biased out of the relay circuit, and hence involved the use of an additional battery and potentiometer. Furthermore, the resistance of the relay was low in comparison with that of the plate circuit. By introducing a step-down transformer between the relay and the plate circuit of the last tube, the additional battery and potentiometer were dispensed with, and higher efficiency was obtained. In the final model of the

apparatus, a full wave copper oxide rectifier was inserted before the relay. This did not require a bias battery and was satisfactory.

The relay is of the Weston model 30 type. A spring contact made of platinum containing 30 per cent iridium 1 inch long, 1/16 inch wide and 0.004 inches thick was substituted for the fixed contact of the relay. This spring contact aids in minimizing disturbances arising from the smaller peaks and ripples that are passed through the amplifier, and in reducing chatter. The primary relay operates a telegraph relay wound to 150 ohms. On this relay, too, an adjustable

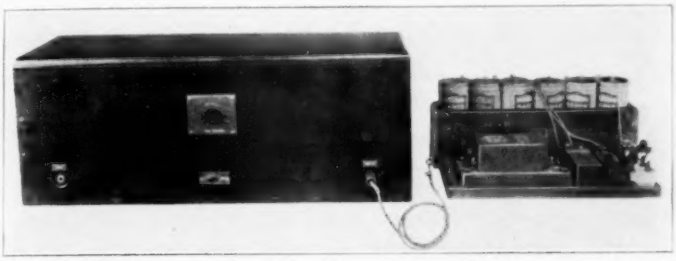

Fig. 3.—The amplifier, relays and counter.

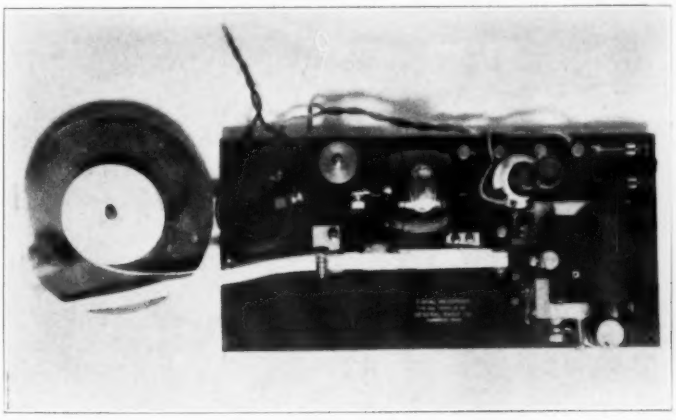

Fig. 4.—The signal recorder.

spring mounted contact was substituted for the fixed contact to avoid rebounding and chatter and consequent false registrations of the counter, and to permit the more rapid action required at high pulse rates.

The Counter.—The second relay operates a telephone message counter. This counter was made more rapid in its action by lightening the armature and shortening its excursion. A contact mounted on this counter is closed at the end of each inward excursion of the armature. This closure actuates the recorder.

The Recorder.—The recorder is a type 256 Signal Siphon Recorder manufactured by the General Radio Company of Cambridge, Mass. (fig. 4). The pen records on a moving tape, each stroke of the pen representing one stroke of the counter, or one heart beat. With proper adjustment, the recorder will not operate unless the counter armature makes a full stroke.

METHOD OF OPERATION

The electrodes are fixed in place over the precordium, one over the apex, the other over the second right intercostal space; the starting switches on the amplifier and the relay are closed, and the gain control on the amplifier is varied until each heart beat is recorded accurately on the counter. Too high

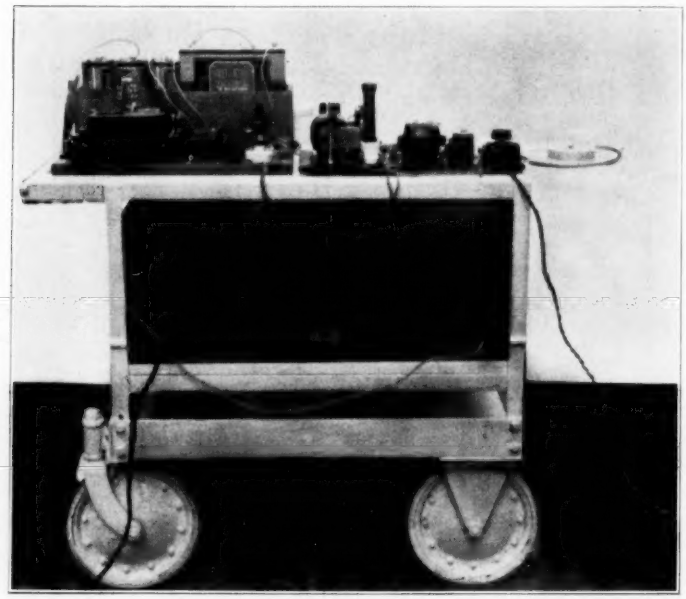

Fig. 5.—Complete apparatus mounted on a cart.

amplification will cause the sensitive relay arm to come up against the stop with such force that it bounds back and makes a double contact, and so registers a false beat on the counter. If this occurs, the spring tension of the sensitive relay should be increased, and if this is not sufficient, the gain should be reduced. The siphon recorder is then thrown into circuit. The number on the counter is read and the time is noted. When the observation of the patient is finished, another reading of the counter is made and the time noted. In this manner an accurate count of the total number of heart beats in the period elapsed is automatically obtained. As a control, the tape of the recorder is inspected, and if it shows no missed or interpolated beats, the count can be accepted as accurate.

COMMENT

If the patient holds his hands and fingers immobile, good records can be obtained by the use of immersion jars filled with salt solution as electrodes. Every movement of the hands or fingers, however, causes a large number of disturbances of the relay. German silver electrodes applied to the arms allow more freedom of motion without readily introducing false signals, and flannel bandages soaked in salt solution and held in place on the arms by many turns of wire are still better. With all of these electrodes, however, unguarded movements of the patient lead to false or missed beats. Thus they defeat the primary purpose of the apparatus—to count the heart beats over long periods of time while the subject follows his ordinary activities. These electrical disturbances are probably due to the action currents of the striated muscles, which obscure the registration of the action current of the heart. Moreover, the water evaporates from the bandages that have been moistened with salt solution. These difficulties led to the use of the small chest electrodes and of green soap as an electrolyte. We have used wires up to 20 feet in length between the electrodes and the input jack of the amplifier, thus allowing the patient full activity.

With these electrodes, free motion of the body and extremities does not cause interference with the regular signaling of the action current of the heart. The subjects can indulge in calisthenic exercises, walk, run, sit down on the floor or lie down and roll from side to side without disturbing the regular registration of the counter, as shown by the graphic record. Coughing and laughing do not introduce inaccuracies. However, if the patient makes all of his muscles tense, as in stretching or straining, serious disturbances in the action of the primary relay and in the whole apparatus are introduced. Apparently the electrical currents accompanying these tetanic muscular contractions are sufficiently large and numerous to obscure the action current of the heart. Mobitz,[3] in attempting to listen to the amplified action current of the heart by means of a telephone receiver, noted a steadily increasing roaring sound when the subject held his breath, and attributed it to the action currents of the tense respiratory muscles—apparently a similar phenomenon. If insufficient amplification is used, change of position of the patient may interfere with the recording and result in the loss of many heart beats. This is probably due to the changing position of the heart and of its electrical axis. Since the chest electrodes are fixed, an alteration in the electrical axis of the heart may cause such a lowering of the voltage to be led off that it is insufficient to operate the primary relay. To avoid this error, it is necessary to employ the maximum amplification con-

3. Mobitz, W.: Ueber Versuche Aktionsströme zu verstärken, Deutsches Arch. f. klin. Med. **149**:209, 1925.

sistent with the regular action of the apparatus and to put the patient through several test exercises before making the final adjustment of amplification gain and of spring tension on the primary relay.

It is essential to insulate the patient during the period of observation, particularly if the floor of the room is a good conductor. We worked in a room with a flooring of magnesite composition and found that whenever the patient, who was placed on a rubber mat, stepped off of the nonconducting rubber, disturbances were apt to arise in the primary relay. The intensity of these disturbances varied from day to day. Similarly, if an uninsulated observer touched the patient, or if the patient touched a radiator or some similar object, the action of the relay became irregular. To insulate the patient, a mat of rubber or other nonconductive material may be placed on the floor. If the patient walks

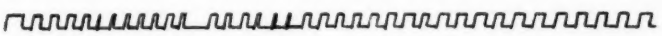

Fig. 6.—False beats caused by the uninsulated observer touching the patient.

Fig. 7.—Record of regular heart beat.

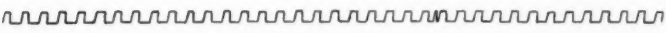

Fig. 8.—Extra beat caused by the uninsulated observer touching the patient.

around, he should wear rubbers. Similarly, the observer, or the nurse, who must touch the patient's bed, should wear rubbers. If the patient is in bed, the bed should be insulated with rubber castors. Similarly, disturbances may arise if the observer touches the magnet of the siphon recorder. Figure 6 shows extra beats caused by the uninsulated observer touching the patient.

The graphic record on the tape acts as a control of the accuracy of the counter when the heart rhythm is regular. At the end of a period of observation, when the counter reading has been noted, the record on the tape is inspected. If all of the signals follow one another without a break, and if there are no irregularly placed interpolated beats, the count may be accepted as correct. In the record of a long observation totaling several thousand beats, five or ten missed beats may be

disregarded as insignificant. However, it is easy to estimate the number of beats that have not been recorded and to allow for them. Extrasystoles can be recognized when they are followed by a compensatory pause. If the record shows many inaccuracies, the count of that particular run must be disregarded.

We are planning to add time signals to the record on the tape so that changes of rate can be determined at all points on the record.

We have tested the cardiotachometer on a large series of patients with normal and abnormal hearts under a great variety of conditions, and have been able to obtain satisfactory readings in all of them. The instrument accurately records heart rates from 40 to 200 a minute. The tape runs at a speed of about 1.2 feet a minute or 72 feet an hour. Although this seems like a long record, its inspection actually takes very little time. In three minutes 72 feet of tape corresponding to one hour's observation can be checked.

Figure 7 is a short strip of the record of case 1. The patient, a girl with simple tachycardia, was under test for forty minutes. During that time, her heart beat 4,740 times, which represents an average rate of 118.5 a minute. The graphic record shows accuracy of the counter with two exceptions, both alike. One of these is shown in figure 8 where an extra interpolated beat was evident. This was caused by the observer touching the patient and was noted at the time it happened. The detailed observations of case 1 are given in table 1. Figure 9 is the record of a patient with mitral stenosis, whose rate was 104 at rest and rose to 200 while she was running in circuit with the cardiotachometer. The record which is continuous and which should be read from left to right and from above downward, shows the absolute accuracy of registration. Figure 10 shows simultaneous cardiograms and tracings of the cardiotachometer of a patient with auricular fibrillation and a heart rate of 118. The records are of different lengths because of the different speed of the two papers. The two tracings correspond absolutely not alone in the total number of beats, but in their relative spacing as well. Figure 11 is a similar tracing of another patient with auricular fibrillation, with a rate of 69. A pulse tracing is substituted for the cardiogram and shows similar correspondence with the cardiotachometer.

Table 1 represents observations on three patients. The sample rate is a casual fifteen second count taken with a watch. The effect of eating is well shown in case 2, a woman with auricular fibrillation. The rate rose from 97 to 124. Case 3 is a record of a girl with mitral stenosis and simple tachycardia. The observations were taken in the evening with the patient in bed.

The possible clinical applications of the cardiotachometer are manifold. Many physiologic problems, such as the response of the heart

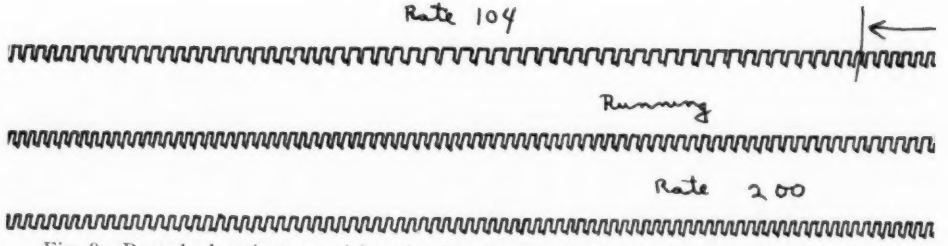

Fig. 9.—Record of patient exercising showing the rate of 104 at rest and a rate of 200 while the patient was running.

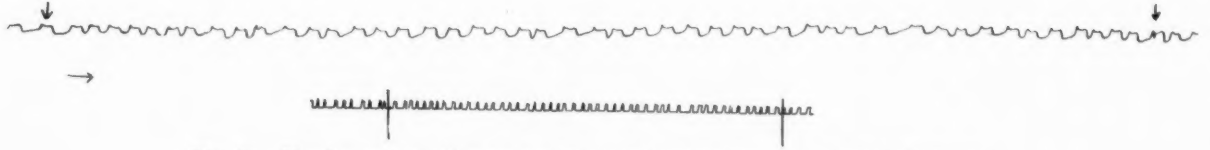

Fig. 10.—Simultaneous cardiogram and cardiotachometer record; auricular fibrillation.

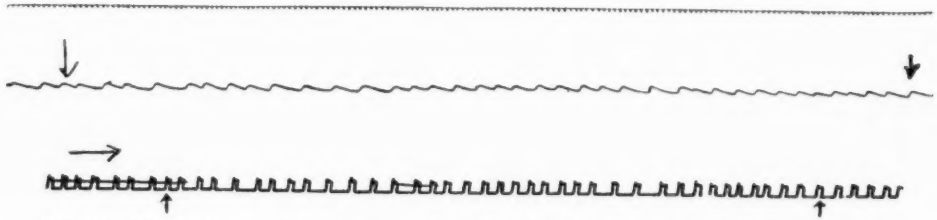

Fig. 11.—Simultaneous sphygmogram and cardiotachometer tracing; auricular fibrillation.

to exercise, drugs, and other stimuli can be studied. The response of the heart during exercise can be accurately measured. As an accessory in many physiologic and clinical examinations, such as basal metabolic rate and minute volume flow determinations, it is invalu-

Record of Heart Beat in Three Patients

(Read up the columns)

	Time	Counter Readings	Total Beats	True Rate	Sample Rate	Remarks
Case 1:	11:42	11,999				
			686	98		
	11:35	11,313				
			1,275	127.5		
	11:25	10,038			128	Half sitting in wheel chair
			717	119.5		
	11:19	9,321			114	Lying in wheel chair
			837	119.6		
	11:12	8,484			128	
			1,225	122.5		
	11:02	7,259			120	Sitting in wheel chair
Total	40 min.		4,740	118.5		
Case 2:	12:26	8,473			100	Sitting quietly
			783	97		
	12:18	7,690			119	Finished dinner
			994	124.2		
	12:10	6,696			112	Began dinner
			683	97.9		
	12:03	6,013			101	
			2,547	94.3		
	11:36	3,466			90	Sitting quietly
Total	50 min.		5,007	100.1		
Case 3:	11:35	19,683			108	Awake
			1,926	96.3		
	11:15	17,757			100	Dozing
			1,958	97.9		
	10:55	15,799			96	Sleeping
			1,970	98.5		
	10:35	13,829			108	Dozing
			2,156	107.8	112	Awake, 10:22
	10:15	11,673			112	Sleeping
			2,113	105.6		
	9:55	9,560			112	Sleeping
			2,151	107.5		
	9:35	7,409			106	Trying to sleep
			2,111	105.5		
	9:15	5,298			108	Trying to sleep
			2,222	111.1		
	8:55	3,076			106	Reading in bed
			2,276	113.8		
	8:35	800				
Total	3 hours		18,883	104.9		

able. The cardiotachometer will give us for the first time an actual count of the total number of heart beats under the conditions and activities of every day life. The device will be of particular value in determining the clinical significance of the simple tachycardias. It is often difficult to ascertain whether a tachycardia is persistent or only transient and due to an anxiety neurosis or to the excitement of the

examination. The cardiotachometer, particularly if it is run while the patient is asleep, will readily distinguish the persistent rapid heart rate that occurs in cases of myocarditis or exophthalmic goiter from a functional acceleration.

SUMMARY

An instrument which has been named the "cardiotachometer" is described, which is actuated by the action current of the heart and records automatically the totality of heart beats over long periods of time.

PLASMA AND CORPUSCULAR CHLORIDES IN PEPTIC ULCER *

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AND
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The work on which this paper is based was done for the purpose of determining whether in cases of peptic ulcer there is a possible relationship between the amount of various chloride constituents of the blood and the amount of free hydrochloric acid secreted in a stated interval at different stages of which blood was withdrawn for examination. While such a relationship was difficult, if not impossible, to discover, other facts of interest and questions arose which it was felt might be of sufficient interest to report.

That there is an increase in both free and total acidity and in the amount of gastric secretion is well known. The results of studies on the blood chlorides in these cases are not so well known, principally because of the small number of studies on this subject. Jordan [1] states that in forty-one cases of ulcer, the average level of whole blood chlorides after-treatment was 570 mg. per hundred cubic centimeters of blood and in ten normal cases 593 mg. Arnoldi [2] found that on a salt poor, meat-free diet there is a tendency for the serum chloride to increase as long as the diet is maintained. Molnar and Hetenyi [3] found that in conditions of hyperacidity the chloride content of the cells and plasma are below normal. We attempted to answer the question whether there is a fundamental difference between normal persons and those with peptic ulcer, by a study of the chloride content of the whole blood, plasma and corpuscles in twenty cases of ulcer in which the diagnosis was made by clinical and roentgenologic methods and

* From the Stomach Study Group and the Otto Baer Fund for Clinical Research of the Michael Reese Hospital, and the Nelson Morris Institute for Medical Research.

* Beds were available for this work through a grant by Mr. Julius Rosenwald.

1. Jordan, Sara M. Calcium, Chloride and Carbon Dioxide Content of Venous Blood in Cases of Gastroduodenal Ulcer Treated with Alkalies, J. A. M. A. **87**:1906 (Dec. 4) 1926.

2. Arnoldi, Walter: Der prozentuale Chlorgehalt des Blutserums bei kochsalzarmer und kochsalzreicher fleischfreier Ernährung sowie bei verschiedener Flüssigkeitszufuhr, Berl. klin. Wchnschr. **50**:675, 1913.

3. Molnar, B., Jr., and Hetenyi, G.: Ueber den Chlorgehalt des Blutserums bei Sekretionsstörungen des Magens und Beinflussung der Anazidität durch Kochsalz., Arch. f. Verdaunngskr. **30**:8, 1922.

in some cases confirmed by operation. Thirteen patients were studied within forty-eight hours after they were admitted to the hospital and before any treatment was given and seven were studied at various periods after treatment was instituted.

METHOD

Ten cubic centimeters of oxalated blood was centrifugalized for ten minutes immediately after it was taken, and the plasma was withdrawn. The chlorides were estimated by the Whitehorn [4] method and recorded in milligrams per hundred cubic centimeters of plasma, whole blood or corpuscles. The corpuscular chloride content was calculated from that of the whole blood and blood plasma. At the end of two weeks similar determinations of the chlorides were made at the start,

TABLE 1.—*Results Attained in the Determinations of the Chlorides Before Treatment*

	Whole Blood			Plasma			Corpuscle		
Case	0 Min.	75 Min.	150 Min.	0 Min.	75 Min.	150 Min.	0 Min.	75 Min.	150 Min.
1	471.9	442.2	.....	559.4	561.0	.....	377.1	313.5	.....
2	455.4	438.9	.....	577.5	554.4	.....	323.1	313.8	.....
10	430.6	412.5	.....	543.0	529.6	.....	308.8	285.6	.....
11	503.3	513.2	.....	589.1	597.3	.....	410.4	422.1	.....
12	564.9	510.8	476.4	621.5	624.2	615.5	503.5	346.4	325.8
15	486.6	435.6	442.2	580.4	595.7	595.7	385.0	262.1	275.8
16	405.9	397.6	420.7	580.8	575.9	589.1	212.3	204.4	238.3
17	438.9	445.5	445.5	599.0	602.3	602.3	265.4	275.6	275.6
18	410.8	396.0	400.9	599.0	544.0	590.7	206.9	235.6	195.2
19	455.4	463.6	466.9	617.1	618.9	612.2	280.2	295.4	309.6
20	438.9	455.4	453.7	590.7	603.9	605.6	274.4	294.0	288.1
21	438.9	427.3	.....	599.0	589.0	.....	265.4	252.1	.....
22	450.4	460.3	457.0	603.9	610.5	608.9	284.2	297.5	292.5
Average	457.9	446.0	445.4	589.3	585.1	602.7	315.2	292.1	275.1

seventy-five minutes and one hundred and fifty minutes after the start of a two and one-half hour period during which fractional tests were made at one-half hour intervals to determine the free and total gastric acidity. The figures of Dodds and Smith [5] in ten normal cases were taken as a standard of comparison in this report. The tables give the results attained in thirteen cases before treatment was begun (table 1); the results when the determinations were made after treatment only (table 2), and when they were made before and after treatment was started (table 3). Table 4 is a statement of the figures of ten normal cases, and table 5 shows the wide variations in individual instances. It can thus be seen from table 5, that the differences in the chloride content before and after treatment are negligible when compared to the

4. Whitehorn, J. C.: Method for Determination of Blood Chlorides, J. Biol. Chem. **45**:449, 1921.

5. Dodds, E. C., and Smith, K. S.: Variations in the Blood Chlorides in Relation to Meals, J. Physiol. **58**:157 (Dec. 28) 1923.

TABLE 2.—*Results Attained in the Determinations of the Chlorides After Treatment*

Case	Whole Blood		Plasma		Corpuscle	
	Before	75 Minutes	Before	75 Minutes	Before	75 Minutes
3	433.9	429.0	541.0	542.9	317.9	305.6
4	603.9	607.2	562.7	557.7	648.8	660.8
5	389.4	417.4	534.6	552.6	232.1	270.6
6	521.4	528.0	618.8	612.2	417.9	436.9
7	442.2	442.2	572.6	567.6	300.8	308.3
8	569.3	572.6	686.4	671.6	442.5	465.4
9	488.4	501.9	622.1	600.1	343.5	401.7

TABLE 3.—*Results Attained in the Determinations of the Chlorides Before and After Treatment*

Case	Whole Blood			Plasma			Corpuscle		
	0 Min.	75 Min.	150 Min.	0 Min.	75 Min.	150 Min.	0 Min.	75 Min.	150 Min.
15	486.6	435.6	442.2	580.4	595.7	595.7	385.0	262.1	275.8
15 repeated	438.9	427.3	430.6	595.7	570.9	599.0	269.0	270.8	246.0
16	405.9	397.6	420.7	580.8	575.9	589.1	212.3	234.4	238.3
16 repeated	430.6	429.0	437.2	605.6	602.3	605.6	241.0	241.2	254.8
17	438.9	445.5	445.5	590.0	602.3	602.3	265.4	275.6	275.6
17 repeated	453.7	421.2	442.2	610.5	579.6	612.2	283.8	249.6	258.1
18	410.8	396.0	400.9	590.0	544.0	590.7	206.9	235.6	196.2
18 repeated	442.2	443.8	438.9	592.4	605.6	589.0	279.6	268.5	265.5
20	438.9	455.4	453.7	590.7	603.9	605.6	274.4	294.0	288.1
20 repeated	430.6	438.9	453.7	570.9	590.0	599.0	278.6	265.5	296.3
21	438.9	427.3	.....	599.0	589.0	.....	265.4	252.1	.....
21 repeated	453.7	445.5	463.6	582.5	608.9	605.6	314.2	298.5	308.7

TABLE 4.—*Chloride Content of Whole Blood, of Plasma and of Corpuscles in Normal Persons* *

	Sodium Chloride in Mg. per 100 Cc.		
	Whole Blood	Plasma	Corpuscle
1	480	605	345
2	560	650	463
3	485	570	393
4	500	785	191
5	480	610	339
6	420	570	242
7	440	580	288
8	475	570	372
9	490	590	382
10	440	565	305
Average	477	609	333

* In the whole blood the figures vary from 420 to 560; in plasma from 565 to 785 and in corpuscles from 191 to 463.

TABLE 5.—*Chloride Content of Whole Blood, of Plasma and of Corpuscles Before and After Treatment*

	0 Min.	75 Min.	150 Min.
Average of chloride content of whole blood before treatment	458	446	445
Average of chloride content of whole blood after treatment	469	469	444
Average of chloride content of plasma before treatment	589	585	603
Average of chloride content of plasma after treatment	592	590	604
Average of chloride content of corpuscles before treatment	315	292	275
Average of chloride content of corpuscles after treatment	336	339	272

individual variations and inconclusive in showing the effect of treatment on the chloride content of the blood constituents. The study of the gastric acidities in these cases failed to show any constant relationship between the chloride content and the acidity curves.

It is interesting to note that the plasma chloride attains the highest level and shows the smallest variations, the corpuscular chloride the lowest level and the largest variations and the whole blood figures in between the other two.

The question arises as to whether alkali treatment produces a disturbance in the chloride reservoir, if such reservoir exists. If there is any change, it is to be found in the tendency of the corpuscular chloride content to increase after treatment. It is interesting to note that in the cases in which a simultaneous study of the gastric juice was made during the periods in which the blood studies were made, the changes, when definitely apparent, occurred in the corpuscles. The corpuscles generally showed a decrease in chlorides at the beginning of the fractional test meal and a return to normal after seventy-five minutes.

The following points are of interest in chloride metabolism:

1. Are the corpuscles the principal chloride carriers? They appear to be, but this cannot be definitely proved.

2. Do the corpuscles supply chlorides as needed and act as a chloride reservoir for the plasma? They appear to do this because of a greater constancy in chloride content of the plasma over the corpuscles and the variability of the chlorides of the corpuscles.

3. Is there some source of supply for the restoration of chlorides? The chloride content of the food intake is low on the diet allowed in the cases cited.

Why should the chloride content remain high and in some instances be higher after treatment? Lim and Ni [6] carried out studies on the blood chloride in animals with a Pawlow pouch and found a diminution in the blood chlorides. Their results, however, are not to be compared with those in human beings, because the constant withdrawal of all the gastric secretion as it accumulated prevented the absorption of any of the chlorides from the gastric juice, whereas in man the secretion is retained. This raises further questions.

4. Do the alkali ions of the alkali administered in any way help to bind chlorides and thereby help to maintain a higher chloride content after treatment than before? At present no attempt is made to answer this question.

5. Does the corpuscular chloride represent more truly the changes that occur in the whole blood? From the fact that the greatest variations occur in the corpuscles, this would appear to be true. However, this is only a conjecture and more experimental work is required to prove or disprove this.

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6. Lim, R. K. S., and Ni, T. S.: Changes in Blood Constituents Accompanying Gastric Secretion: I. Chlorides, Am. J. Physiol. **75**:475 (Jan.) 1926.

CONCLUSIONS

1. The results obtained in normal persons and in patients with peptic ulcer do not show great differences.

2. The plasma chloride content reaches the highest level and shows the smallest variations.

3. The corpuscular content attains the lowest level and is less constant.

4. The changes in the corpuscular content suggest that the corpuscles are important in chloride metabolism.

5. No close relationship between the blood chlorides and the gastric acidity is noticed.

EFFECT OF TOXEMIA ON TOLERANCE FOR DEXTROSE AND ON THE ACTION OF INSULIN *

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In a previous communication, I [1] presented data showing the effect of toxemia on the tolerance for dextrose of rabbits intoxicated with diphtheria toxin. Daily tests for tolerance for dextrose were made on the animals during the course of their toxemia, and a progressive decrease in the tolerance for sugar was noted.

The series of observation to be presented in this article were made on rabbits that were treated as described and, in addition, they received uniform doses of insulin each time dextrose was administered. It was thought that by this procedure some analytic information might be obtained as to the effects of toxemia on the tolerance for dextrose and on the action of insulin.

Four rabbits were used, one as a control. All food was withdrawn from the cages, but water was allowed, and a single injection of 0.0075 cc. of diphtheria toxin [2] was given subcutaneously to each animal except the control. On the following day, or twenty-four hours later, tests of the tolerance for dextrose were begun and were made daily on each animal until its death. Five grams of dextrose in 25 cc. of water was given by stomach tube. Two units of insulin [2] were given daily, fifteen minutes after the administration of the dextrose. Samples of blood for determination of the sugar content were drawn from the marginal veins of the ear before the administration of dextrose and thirty, sixty and one hundred and twenty minutes following its administration. All of these determinations were made according to the Folin-Wu method. Of the three toxic animals, one lived six days, one seven and the third eight days. The control animal was fed at the conclusion of the experiment and did not show any untoward effects of the starvation.

The results of the daily tests are tabulated in tables 1, 2, 3 and 4 and are presented graphically in charts 1, 2, 3 and 4.

There is one outstanding observation in these experiments. Examination of the curves shows that the two units of insulin given each day,

* From the Departments of Internal Medicine and Physiology, Baylor University, College of Medicine.

1. Sweeney, J. S., and Lackey, R. W.: The Effect of Toxemia on Tolerance for Dextrose, Arch. Int. Med. **41**:257 (Feb.) 1928.

2. I am indebted to the Eli Lilly Company, who kindly supplied the diphtheria toxin and insulin used in this experiment.

fifteen minutes after the administration of the dextrose, had a persistent and fairly uniform action in lowering the postprandial blood sugars in the presence of an increasing toxemia. It will be noted that the two hour determinations in two or three instances were not materially lower than the one hour readings. In one instance there was an actual rise at the two hour interval, but this increase was slight. I [1] have recently shown that daily tests to ascertain the tolerance for dextrose of rabbits that were made toxic with diphtheria toxin, but that did not receive insulin, showed that there was an increasing inability to remove

TABLE 1.—*Results of Daily Tests of Tolerance of Rabbit 4 for Dextrose* *

Hours of Fasting and Toxemia	Temperature (F.) Rectal	Blood Sugar, Mg. per 100 Cc.			
		Fasting	After Dextrose		
			30 Minutes	60 Minutes	120 Minutes
24	104.3	114	179	171	114
48	104.1	115	160	180	157
72	103.2	118	182	244	198
96	103	118	200	217	163
120	102.8	123	241	282	235
144	103	154	259	306	306
168	102.8	145	278	279	260
192	.....	154	339	400	400

* The animal received 0.0075 cc. of diphtheria toxin. Two units of insulin were given subcutaneously each day fifteen minutes after the administration of dextrose.

TABLE 2.—*Results of Daily Tests of Tolerance of Rabbit 5 for Dextrose* *

Hours of Fasting and Toxemia	Temperature (F.) Rectal	Blood Sugar, Mg. per 100 Cc.			
		Fasting	After Dextrose		
			30 Minutes	60 Minutes	120 Minutes
24	104.5	114	187	177	112
48	102.8	131	200	198	168
72	102.1	110	183	256	244
96	102.5	95	189	233	222
120	101.9	139	241	308	308
144	102.8	154	286	364	377
168	103.4	139	278	323	308

* The animal received 0.0075 cc. of diphtheria toxin. Two units of insulin were given subcutaneously each day fifteen minutes after the administration of dextrose.

dextrose from the blood. In fact, there appeared to be a quantitative relationship between the intensity of the toxemia and the impairment of the rabbits' tolerance for sugar. With these observations in mind, it requires only casual inspection of the charts accompanying this article to see that the toxemia does not cause an appreciable change in the action of the injected insulin; in other words, as stated before, there seems to have been a consistent "two unit action" throughout these experiments, even in the presence of an increasing toxemia.

COMMENT

In order to suggest an explanation for the phenomenon described and other related phenomena of the effect of toxemias on the tolerance

for dextrose, it is necessary to consider the factors involved in the production of the so-called normal tolerance curve. I [3] have previously suggested that the ingestion of dextrose stimulates the production of sufficient insulin to cause the disappearance of the excess blood sugar within two or three hours. This effect of the ingested dextrose has been suggested by others.[4] Jordan [5] has more recently presented some experimental work that supports this view. It was further suggested that the

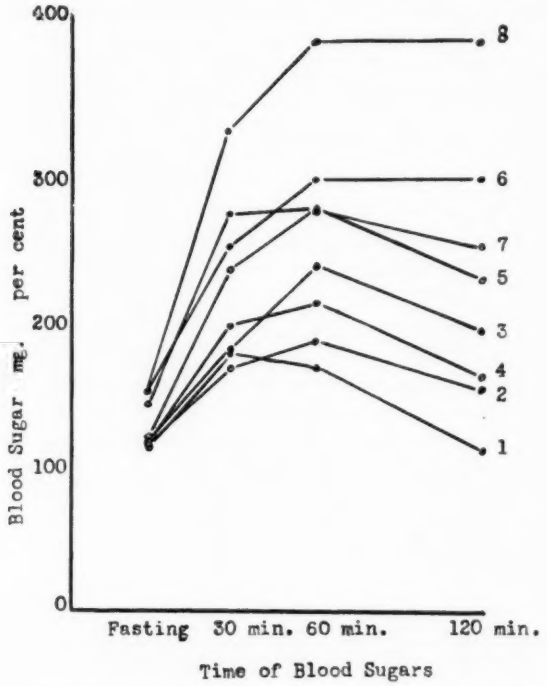

Chart 1.—Daily curves showing tolerance of rabbit 4 for dextrose. The animal received 0.0075 cc. of diphtheria toxin. Two units of insulin were given subcutaneously each day fifteen minutes after the administration of dextrose. The figures at the end of each curve represent the number of days of fasting and toxemia.

3. Sweeney, J. S.: Dietary Factors That Influence the Dextrose Tolerance Test, Arch. Int. Med. **40**:818 (Dec.) 1927.

4. Sevringhaus, E. L., and Smith, M. E.: Science **61**:92, 1925. Thalhimer, W. F.; Raine, Forrester; Perry, M. C., and Buttles, J.: The Effect of Injections of Dextrose and of Insulin and Dextrose Upon Blood Sugar, J. A. M. A. **87**:391 (Aug. 7) 1926. Lawrence, R. D.: Quart. J. Med. **20**:77, 1926.

5. Jordan, E. M.: Am. J. Physiol. **80**:441, 1927.

excess of blood sugar was largely stored as glycogen. This hypothesis is consistent with the more recent views regarding the mode of action of insulin.[6] I am familiar with the somewhat discordant experimental results bearing on this subject. For a more comprehensive analysis of this phase of the subject, the reader is referred to the excellent discussion of Barbour, Chaikoff, Macleod and Orr [6] on their experimental results.

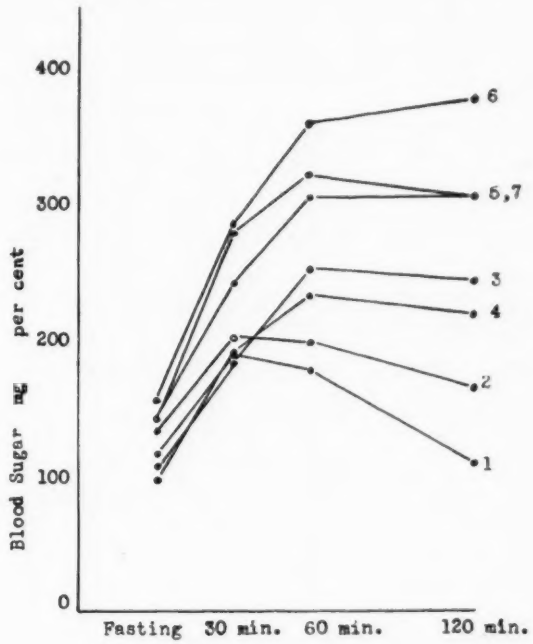

Chart 2.—Daily curves showing tolerance of rabbit 5 for dextrose. The animal received 0.0075 cc. of diphtheria toxin. Two units of insulin were given subcutaneously each day fifteen minutes after the administration of dextrose. The figures at the end of each curve represent the number of days of fasting and toxemia.

I [3] have previously demonstrated that persons who have been fed on rich carbohydrate diets for two days prior to the performance of a test of their tolerance for dextrose have shown a marked increase in

6. Rabinowitch, I. M., and Bozin, Eleanor V.: Brit. J. Exper. Path. **8**:4, 1927. Barbour, A. D.; Chaikoff, I. L.; Macleod, J. J. R., and Orr, M. D.; Am. J. Physiol. **80**:243, 1927. Macleod, J. J. R.: Physiology and Biochemistry in Modern Medicine, 5, St. Louis, C. V. Mosby Company, 1926.

tolerance. Persons who have been starved or who have been fed on exclusive fat or protein diets have shown a definitely decreased tolerance for dextrose. The effect of diet and fasting on tolerance for carbohydrates has been referred to by other investigators.[7] To explain these results, I have suggested that there was a more active stimulation of the production of insulin in those who had received carbohydrate feedings which resulted in a quick removal of the excess dextrose from the blood. Those who had been fed on fats or proteins or who had been

TABLE 3.—*Results of Daily Tests of Tolerance of Rabbit 6 for Dextrose* *

Hours of Fasting and Toxemia	Temperature (F.) Rectal	Blood Sugar, Mg. per 100 Cc.			
		Fasting	After Dextrose		
			30 Minutes	60 Minutes	120 Minutes
24	102.6	118	182	123	87
48	102.7	111	286	329	299
72	103.4	145	282	274	182
96	102.6	111	238	227	189
120	103.6	137	220	217	168
144	106	161	250	230	†

* The animal received 0.0075 cc. of diphtheria toxin. Two units of insulin were given subcutaneously each day fifteen minutes after the administration of dextrose.
† Animal died during the one hour interval.

TABLE 4.—*Results of Daily Tests of Tolerance of Rabbit 7 for Dextrose (Control)* *

Hours of Fasting	Temperature (F.) Rectal	Blood Sugar, Mg. per 100 Cc.			
		Fasting	After Dextrose		
			30 Minutes	60 Minutes	120 Minutes
24	101.7	101	180	150	80
48	102.1	102	169	179	132
72	103.7	140	179	145	114
96	103.8	118	148	114	68
120	103.8	129	204	171	92
144	103.4	56	89	64	58

* Diphtheria toxin was not given. Two units of insulin were given subcutaneously each day fifteen minutes after the administration of dextrose.

starved had had little if any foods (carbohydrates) that stimulated the production of insulin, and when dextrose was ingested the production of insulin was delayed, which resulted in a sluggish removal of the excess dextrose from the blood.

It is difficult to explain the foregoing phenomena in any way except by the action of endogenous insulin. If increased or decreased oxidation of the dextrose was the cause, one would have to account for the

7. Kageura, N.: J. Biochem. **1**:333, 1922. Kageura, N.: J. Biochem. **1**:389, 1922. Greenwald, I.; Gross, J., and Samet, J.: J. Biol. Chem. **62**:401, 1924. Southwood, A. R.: M. J. Australia **10**:460, 1923. Staub, H.: Ztschr.f.klin.Med. **93**:89, 1922. Traugott, K.: Klin. Wchnschr. **1**:892, 1922. Du Vigneaud, Vincent; and Karr, W. G.: J. Biol. Chem. **66**:1, 1925.

effect of the antecedent diets on this process. I cannot think of any reason why antecedent diets rich in sugars should cause a more rapid combustion of dextrose than diets consisting of fat or protein. The only other explanation apparent would be that there is a more rapid elimination of the excess sugar through the kidneys. This, however, cannot be held to be the explanation, because there was no postprandial sugar in the urine of the persons who had previously received the carbohydrate feedings.

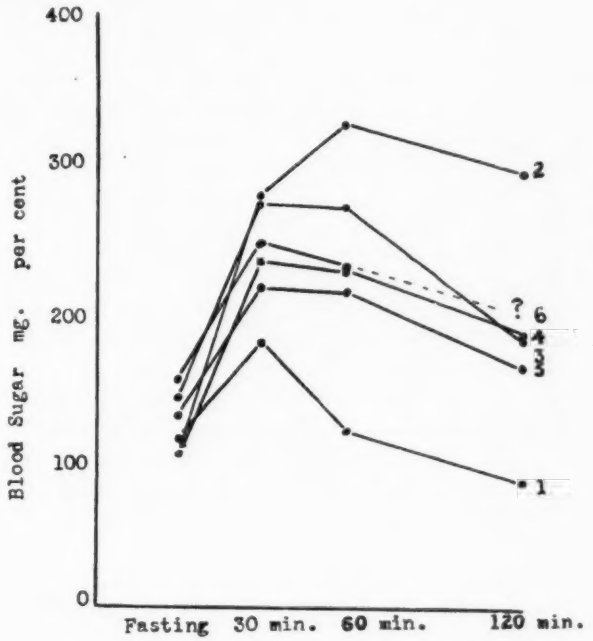

Chart 3.—Daily curves showing tolerance of rabbit 6 for dextrose. The animal received 0.0075 cc. of diphtheria toxin. Two units of insulin were given subcutaneously each day fifteen minutes after the administration of dextrose. The figures at the end of each curve represent the number of days of fasting and toxemia.

In another series of experiments referred to earlier in tnis paper, I [1] showed that there was a progressive decrease in tolerance for sugar by rabbits in the presence of an increasing toxemia. That toxemia causes a decrease in tolerance for sugar has long been known. Two theories have been suggested to explain this effect. Tisdall and his co-workers [8]

8. Tisdall, F. F.; Drake, T. G. H., and Brown, Olan: Production of Lowered Carbohydrate Toleration in Dogs, Am. J. Dis. Child. **32**:854 (Dec.) 1926.

suggested that it is a result of an increase in the glycogenolytic function of the body or an impairment of the ability of the organism to store carbohydrates. Lawrence and Buckley [9] have suggested that it is due to an increased glycogenolysis resulting from the stimulation of the "'thyro-adrenal apparatus." On the basis of what has been said in the preceding paragraphs, I am more inclined to explain the effect of toxemia by an impairment of the animals' ability to store carbohydrates, which is but another way of saying that there is an impairment of glycogenesis. It is

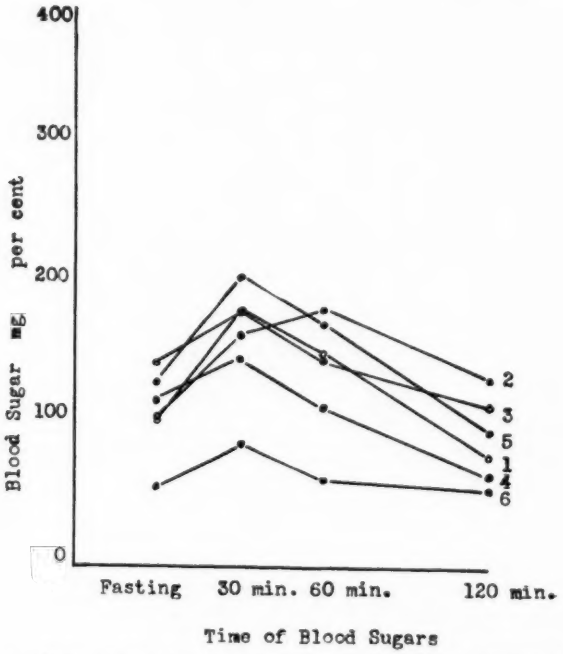

Chart 4.—Daily curves showing tolerance of rabbit 7 for dextrose (control). Diphtheria toxin was not given. Two units of insulin were given subcutaneously each day fifteen minutes after the administration of dextrose. The figures at the end of each curve represent the number of days of fasting.

difficult to believe that glycogenolysis alone can account for the daily, progressively abnormal postprandial blood sugars observed in the toxic rabbits to which I have referred. If glycogenolysis was the cause, it would appear that dextrose itself might be stimulating that process, because the postprandial blood sugars rise to materially higher levels each day. I believe that the tendency for the blood sugars to be higher each day in fasting toxic animals is probably a result of glycogenolysis.

9. Lawrence, R. D., and Buckley, O. B.: Brit. J. Exper. Path. **8**:1, 1927.

If it may be assumed, then, that the effect of toxemia is principally that of an impairment of an animal's ability to store carbohydrates, and if the storage of carbohydrates is brought about by endogenous insulin, it may be suggested that the toxemia has a direct depressing effect on the production of insulin. The experiments contained in this paper have demonstrated that the action of injected insulin is affected little if at all in toxic animals. The logical deduction, therefore, is that toxemia suppresses the endogenous production of insulin. From the experimental data at hand, this explanation seems to me to be more plausible than that of an increased glycogenolysis or a disturbed glycogenesis independent of endogenous insulin.

The hypothesis just outlined is consistent with certain clinical phenomena. For example, on this basis the onset of diabetes during or following acute infections might be explained. The occurrence of diabetes during pregnancy might be explained in like manner. The mechanism involved in such cases would be suppression of insulin production from an inherently weakened pancreas, due to toxemia. More and more attention is being paid to the rôle that heredity plays in the etiology of diabetes.[10] The variation in the doses of insulin required to control diabetes in those suffering from infectious processes may be an expression of the toxic effects on the remaining functioning islands of Langerhans.

SUMMARY AND CONCLUSIONS

Daily tests of the tolerance for dextrose were made on rabbits in which toxemias had been produced with diphtheria toxin. Two units of insulin were injected daily fifteen minutes after the administration of dextrose. The toxemia appeared to have little, if any, effect on the injected insulin. On the basis of these and preceding experiments, it is suggested that the effect of toxemia is that of a suppression of endogenous production of insulin.

10. Von Noorden, C.: Die Zuckerkrankheit und ihre Behandlung, ed. 8, Berlin, Julius Springer, 1927.

THE ICTERUS INDEX

SPECTROPHOTOMETRIC AND QUANTITATIVE STUDIES *

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The two quantitative clinical methods now available for estimating the degree of bilirubinemia are (1) the van den Bergh and (2) the icterus index. The latter, although simple, has the disadvantage in that the degree of jaundice is expressed in arbitrary units of icterus index, instead of in milligrams of bilirubin, one unit being the color equivalent of a 1 : 10,000 aqueous solution of potassium dichromate. It has been our aim in this work to inquire into the basic colorimetric relationships between potassium dichromate and various solutions of bilirubin and to assign, if possible, a definite value in milligrams of bilirubin to the dichromate dilutions. We have also investigated the effect of small amounts of hemolysis.

Murphy [1] and Davis [2] have adequately reviewed the history of the icterus index. It has long been assumed that bilirubin was the pigment responsible for jaundice, as spectrophotometric analyses of jaundice serums correspond with the analyses of bilirubin made by Vierordt [3] in 1876. Three years previously, he had published the results of analyses on serum showing an absorption band which was in the same region as bilirubin.[4] Sheard and his co-workers [5] have also compared jaundiced serums with solutions of bilirubin in serum and have found them to be identical. We agree with these workers on the whole, although several of their statements will be discussed in the course of this paper.

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1. Murphy, Wm. P.: An Easy Method of Estimating the Amount of Jaundice by Means of Blood Serum, Boston M. & S. J. **194**:297 (Feb. 18) 1926; Biliary System Function Tests, Arch. Int. Med. **37**:797 (June) 1926.

2. Davis, D.: The Determination of Icterus Index of Capillary Blood, Am. J. M. Sc. **172**:848 (Dec.) 1926.

3. Vierordt, Karl: Die quantitative Spectralanalyse in ihrer Anwendung auf Physiologie, Physik, Chemie und Techologie, Tubingen, H. Laupp, 1876.

4. Vierordt, Karl: Die Anwendung des Spectralapparates zur Photometrie des Absorptionspektren und zur quantitative chemischen Analyse, Tubingen, H. Laupp, 1873.

5. Sheard, C.; Mann, F. C., and Bollman, J. L.: Spectrophotometric Determinations of Purified Bilirubin, Am. J. Physiol. **81**:774 (Aug.) 1927. Magath, T. B., and Sheard, C.: Spectrophotometric Analysis of Blood Serum in Normal and Pathologic Conditions, Arch. Int. Med. **39**:214 (Feb.) 1927. Sheard, C.; Baldes, E. J.; Mann, F. C., and Bollman, J. L.: Spectrophotometric Determinations of Purified Bilirubin, Am. J. Physiol. **76**:577 (May) 1926.

METHOD

For the purified bilirubin studied in this work we are indebted to Dr. Hans Clarke in charge of the department of synthetic chemistry of the Eastman Kodak Company. A short description of the method of preparation is found in the paper of Sheard, Mann and Bollman.[6]

For the greater part of the studies on the quantitative analyses of the various solutions, we have used a Bausch and Lomb spectrophotometer. Kennedy,[7] from this laboratory, has recently described this particular instrument in detail. The photometer is of the Martens type with extinction points at 0 and 90 degrees and a match point close to 45 degrees. A slight constant deviation of the latter does not make any difference, as all of our solutions were examined first in one field and then in the other, and a sum of the two readings was taken. This instrument covers the visual range adequately. However, as our work has been with absorption bands in the violet end of the spectrum, where accurate visual readings are difficult, added accuracy has been obtained through the frequent employment of photographic records of the absorption bands. These records were obtained on a Hilger quartz spectrograph, the property of the Research Laboratory of the Eastman Kodak Company through the courtesy of Dr. C. E. K. Mees, director of the laboratory, and the efforts of Messrs. E. E. Richardson and Floyd Hertle, who have done the analytic work.

POTASSIUM DICHROMATE

Our stock solution of potassium dichromate was prepared by dissolving 10 Gm. of chemically pure crystals in a liter of distilled water. To this solution 2 drops of concentrated sulphuric acid were added, as recommended by Meulengracht.[8] We believe this to be of importance, as a slight amount of alkali changes the dichromate to chromate with a corresponding change in color, the latter being more yellow and thus leading to considerable error. This change is particularly apt to go unnoticed in the more dilute solutions. Chart 1 expresses graphically the absorption of 1 Gm. of potassium dichromate per liter, with 2 drops of sulphuric acid per liter and also the absorption of the same dilution of dichromate with 1 drop of concentrated sodium hydroxide to 100 cc. of solution. This dilution of dichromate, which has an icterus index of 10, has a rather low optical density in the visual region; nevertheless, the curve of the absorption band is extremely steep in the region in which we are interested, from 460 to 430 millimicrons, and from the chart it will be seen that the maximum absorption is in the ultraviolet end of the spectrum, where this dilution gives an optical density greater than 2.3, the limit at which the Hilger spectrograph was set.

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6. Sheard (footnote 5, first reference).

7. Kennedy, R. P.: A Spectrophotometric Study of Blood Solutions, Am. J. Physiol. **79**:346 (Jan.) 1927.

8. Meulengracht, E.: Ein Bilirubinkolorimeter behufs klinischer Bestimmung der Bilirubinmenge im Blute, Deutsches Arch. f. klin. Med. **137**:38 (Aug. 12) 1921.

It will be seen that the addition of alkali has definitely changed the color, since the absorption band is moved toward the ultraviolet. As we should expect, this corresponds with the absorption band of potassium chromate. Once prepared, the dichromate keeps well, for there was not any perceptible change when it was kept in the dark for two months or when it was exposed to the light for a similar length of time.

BILIRUBIN

The chemical composition of bilirubin is still uncertain, but it probably has the formula $C_{32}H_{36}N_4O_6$, containing two carboxyl groups which make it a weak acid.[9] The sodium salt is much more soluble than the acid itself.

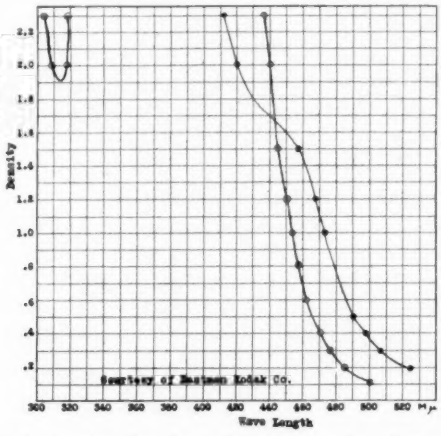

Chart 1.—Graph showing the absorption of potassium dichromate. The line with the dark circles indicates the absorption of 1 Gm. per liter of potassium dichromate with 2 drops of sulphuric acid per liter; the line with the light circles indicates the absorption of 1 Gm. per liter of potassium dichromate with 1 drop of sodium hydroxide to 100 cc. of solution.

The purified bilirubin with which we worked was insoluble without the addition of alkali in the serum of both man and dog. As we were not certain that bilirubin occurs in nature as the salt or that the salt and the acid are photometrically identical, a number of different solvents were tried. These included: chloroform, absolute ethyl alcohol, acetone, a mixture of alcohol and acetone 3:1, the same mixture made alkaline, various aqueous buffer solutions and dog serum. The observations with these solvents are summarized.

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9. Orndorff, W. R., and Teeple, J. E.: On Bilirubin, the Red Coloring Matter of the Bile, Am. Chem. J. **33**:215 (March) 1905.

Bilirubin in Chloroform.—The quality of chloroform used was that generally described as "for anesthesia" and put up in brown glass bottles. No difference was noted between Squibb's and Mallinckrodt's, the two preparations used. Fifty milligrams of bilirubin was completely dissolved in 200 cc. of chloroform at the end of three days in the incubator at a temperature of 37.5 C. If the solution was kept in the icebox, the time required for the bilirubin to dissolve was somewhat longer. This stock solution was kept in dark brown, glass-stoppered bottles, and sealed with paraffin. As the stock solution was too dense for spectrophotometric examination, various dilutions were made containing from 8 to 40 mg. per liter, the optimum for analysis being 20 mg.

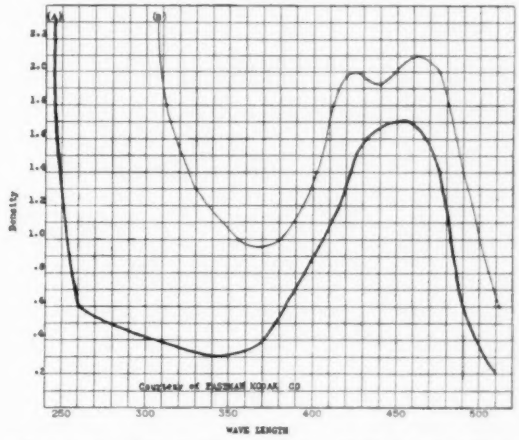

Chart 2.—Curves showing *A* the absorption of 20 mg. per liter of bilirubin in chloroform and *B* the absorption of 16 mg. per liter of bilirubin in dog serum. The stock solution of bilirubin in serum contained 25 mg. of bilirubin in 50 cc. of serum, 25 cc. of hundredth-normal sodium hydroxide and 25 cc. of hundredth-normal hydrochloric acid. The dilution contained 16 mg. per liter and 96.8 per cent serum density of 0.55 at 450 millimicrons.

A chloroform solution of bilirubin contains two absorption bands, one in the visible spectrum and one in the ultraviolet. There is almost complete transmission of red, orange and yellow light and some transmission of green light. Beginning at a wave length around 520 millimicrons in the region of the green, however, it rapidly absorbs increasing amounts of green and blue rays, the maximum absorption being around 450 millimicrons. The absorption decreases throughout the violet and into the ultraviolet, the maximum transmission occurring in the region of 350 millimicrons, when at first gradually and then more sharply the absorption rises to a maximum in the region of 250 millimicrons. The curve is shown in chart 2. This curve is not in accord with Vierordt's

observations on a visual instrument, as he obtained increasing densities up to 430 millimicrons [3] and even at 410 millimicrons [10] where his curve ends. Even at 450 millimicrons it is difficult to make comparisons. This difficulty increases markedly the nearer the ultraviolet is approached, until at 420 millimicrons readings are, in our experience, impossible, due to the physiologic limits of the eye. The relative visibility of the human eye has been accurately determined for the different wave lengths. At 450 millimicrons it is approximately 3.8 per cent of that at the maximum, which is at 556 millimicrons; at 420 millimicrons it is only 0.4 per cent of the maximum.[11] We have never tried to read below 440 millimicrons, and for 440, 450 and 460 millimicrons, we have taken the average of a number of readings. It has not been uncommon to find a maximum deviation of optical density at 450 millimicrons of from 5 to 6 per cent from the mean. It is obvious that the photographic records for the spectral region from 400 to 440 millimicrons constitute the most accurate method available. For one other reason we believe our visual readings are more accurate than those of Vierordt. With our instrument we have been able to work with an ocular shutter width such that the field we are examining at 450 millimicrons is only from 0.8 to 1 millimicron in width. Judging by certain of Vierordt's charts, we believe that from this region to 430 millimicrons he was working with a field from 5.5 to 34 millimicrons in width. Obviously, when examining the edge of an absorption band where the slope is steep, the narrower the field the more accurate the determination.

For every true solution there is a quantitative relationship between the absorption of light and the concentration of the solution. The factor which expresses this quantitative relationship is usually designated as the absorption ratio "A" which is a constant for a particular substance in the same solvent at a given wave length. The relationship may be expressed by the equation $C = A \times \frac{D}{H}$ in which C is the molecular concentration; A, the absorption ratio; D, the optical density (logarithm of opacity, opacity being the reciprocal of the transmission) and H, the thickness. In all of our readings we have used a cell of uniform thickness, 1 cm. Therefore, $H = 1$ and $C = A \times D$ and $A = \frac{C}{D}$.

Using this formula and expressing "C" in milligrams per liter, we have determined the ratio of absorption for bilirubin in chloroform at 450 millimicrons, and have found it to be 11.4, this being an average of the readings of four different observers in this laboratory during the course of sixteen months. All observations were made on freshly pre-

10. Krüss, Gerhard and Hugo: Kolorimetrie und quantitative Spekralanalyse, Leipsic, Leopold Voss, 1909.

11. Illuminating Engineering Nomenclature and Photometric Standard Transaction, Eng. Soc. **20**:632 (July) 1925.

pared chloroform solutions of purified bilirubin with concentrations varying from 8 to 20 mg. per liter. There is a maximum deviation from the mean of 9.5 per cent and an average deviation of 5.5 per cent. Considering the part of the spectrum in which we are working, we do not regard this as excessive.

In table 1 our determinations are compared with those obtained from two other sources. The first are the ratios computed from the readings furnished us by the Eastman Kodak Company obtained on their quartz spectrograph. Two observations each gave a ratio of 11.77. The second set of figures are by Vierordt quoted by Krüss.[10] It is

TABLE 1.—*Absorption Ratios of Bilirubin in Chloroform*

Observer	Date	Mg. per Liter	Wave Length	D	A = C/E
R. P. K.	3/10/20	10	449.5 - 450.5	0.882	11.35
W. W.	4/ 7/27	20	449.5 - 450.5	1.73	11.56
E. E.	6/28/27	20	449.5 - 450.5	1.935	10.35
E. E.	6/28/27	10	449.5 - 450.5	0.829	12.05
E. E.	6/28/27	15	449.5 - 450.5	1.192	12.60
E. E.	6/28/27	8	449.5 - 450.5	0.655	12.22
C. C.	7/ 7/27	20	449.5 - 450.5	1.854	10.77
C. C.	7/11/27	20	449.5 - 450.5	1.816	11.00
E. E.	7/12/27	20	449.5 - 450.5	1.918	10.42
E. E.	7/13/27	20	449.5 - 450.5	1.710	11.67
Mean value for A					11.40
Maximum deviation from mean for A					9.5 per cent
Average deviation from mean for A					5.5 per cent
E. K. Co.	7/28/27	20	Approx. 450	1.700	11.77
E. K. Co.	7/29/27	20	Approx. 450	1.700	11.77
Mean value for A					11.77
Vierordt	1876	5	437 - 450	0.475	10.53
Vierordt	1876	5	450 - 462	0.469	10.67
Vierordt	1876	12.5	450 - 462	.....	12.23
Mean value for A					11.14
Maximum deviation from mean for A					9.79 per cent
Average deviation from mean for A					6.48 per cent

D indicates the optical density (negative logarithm of transmission); C, the concentration in milligrams per liter; E, the extinction coefficient (equal to D when thickness of cell containing solution is 1 cm.) and A, the ratio of absorption (equal to C/D).

rather difficult to compare these figures accurately as they have to be correlated with our readings of the wave length. The field examined was wide, and the ratio differed markedly for different concentrations. As we have averaged them, however, the ratio of absorption is 11.14 with a maximum deviation of 9.79 per cent.

Instability of Chloroform Solution of Bilirubin.—We have had a concentrated solution of bilirubin in chloroform (50 mg. per hundred cubic centimeters) in an icebox for seventeen months. It was kept in a brown, glass-stoppered bottle. During that time it had faded approximately 20 per cent as judged by single dilutions prepared at each end of the period. We have kept dilute solutions 20 mg. per liter in the dark for six weeks, and have found at the end of two weeks that the readings were identical within the limits of error, while at the end of four and

six weeks there was definite fading of from 7 to 10 per cent. Richardson [12] said that he had detected obvious fading in such a solution in three days when kept in the dark. When exposed to the light, even subdued light, the color fades rapidly. It has been our experience that when a tightly corked test tube containing a solution of 20 mg. per liter is left in the light of a northern window on a bright day, where there are no direct sun rays, the color will have faded so much in six hours that it is impossible to make a spectrophotometric analysis. The same solution, which showed a fading of only 7 per cent when kept in the dark for six weeks, showed a fading of 12 per cent of the remainder when left in the beam of the spectrophotometer for four hours, the source of light being a 250 watt bulb. It is obvious that analyses on freshly prepared solutions of bilirubin should be made quickly, and that at all other times the bilirubin should be kept in the dark.

Acetone.—Five milligrams of bilirubin were partially dissolved in 100 cc. of acetone at the end of one week at room temperature. There was no more in solution at the end of six weeks. The curve resembled the chloroform curve. The maximum optical density occurred at 450 millimicrons and, with a 1 cm. layer, averaged 0.67.

Absolute Alcohol.—Five milligrams of bilirubin remained practically undissolved in 100 cc. of absolute ethyl alcohol at the end of six weeks. There was insufficient color in the solute to permit an analysis. It is to be remembered that in the preparation of purified bilirubin Orndorff and Teeple [9] found that it was insoluble in absolute alcohol.

Absolute Alcohol, Alkaline.—To the foregoing solution we added 1 cc. of tenth-normal alcoholic sodium hydroxide (the hydroxide being dissolved in 95 per cent alcohol.) The bilirubin immediately went into solution. It was diluted to 20 mg. per liter, and a spectrophotometric analysis was made. The point of maximum absorption was still at 450 millimicrons, but the curve did not seem so steep as that when chloroform was used. The optical density, too, was only 1.17, much less than any equivalent concentration in chloroform.

Alcohol (3 parts of 95 per cent) — Acetone (1 part) Mixture.—Twenty-five milligrams of bilirubin remained practically undissolved in 100 cc. of alcohol-acetone mixture for three days. Owing to the turbidity of the solution, curves were not made.

Alcohol-Acetone, Alkaline.—To the foregoing mixture we added 1 cc. of tenth-normal alcoholic sodium hydroxide. The bilirubin immediately dissolved. This alkaline mixture was then diluted with the stock alcohol-acetone mixture to a concentration of 10 mg. per liter, and a

12. Richardson, E. E.: Research Laboratory Eastman Kodak Company, personal communication to the authors.

curve was made. Like the alkaline alcoholic curve, the optical density was not so great as when an equivalent chloroform dilution was used, and the curve was not so steep.

Aqueous Solutions.—Five milligrams of bilirubin were practically undissolved in distilled water at the end of a week. It immediately went into solution on the addition of a few drops of concentrated sodium hydroxide. Various buffer solutions were made, and several rough colorimetric calculations of p_H were made according to the method of Clark and Lubs.[13] It was found that bilirubin would quickly dissolve in a weak solution of sodium carbonate, with a p_H unknown but greater than 9.8. It remained undissolved in a solution of disodium phosphate with a p_H around 9.6.

These solutions were much more unstable colorimetrically than the solutions in chloroform. When test tubes containing these dilutions were brought into daylight in test tube racks so constructed that the lower portion of the tube was partially protected from the light, it was found that the portion of the solution exposed to the light had become practically colorless at the end of two hours, while the solution in the lower portion of the tubes still contained much of its color.

DYES

In view of the fact that the curves showing the absorption of bilirubin and potassium dichromate are obviously different, an attempt was made to find a yellow dye which would more nearly resemble bilirubin. Aqueous solutions of quinoline yellow, fast yellow, flavazine and chrysoidine Y were prepared. The absorption spectra of all of these with the exception of chrysoidine Y differed markedly from that of bilirubin. On theoretical grounds chrysoidine should be fairly satisfactory, as its maximum absorption occurs in the region of 450 millimicrons the same as bilirubin and the curve is fairly sharp, but unfortunately the aqueous solution does not match jaundiced serum nearly so well as potassium dichromate, which, despite its theoretical disadvantages, works surprisingly well and will probably continue as a standard until a better one has been developed.

BILIRUBIN DISSOLVED IN DOG SERUM

As this problem is particularly concerned with bilirubinemia, it was thought advisable to measure the color or to determine the absorption curve of definite known amounts of bilirubin in known amounts of serum. A serum which does not contain any pigments would be ideal for this purpose. As dog serum contains comparatively little and is easily available, it was used.

13. Clark, W. M.: The Determination of Hydrogen Ions, Baltimore, Williams & Wilkins Company, 1922.

Healthy stock dogs were used, and from 100 to 300 cc. of blood was drawn by suction directly into 100 cc. centrifugal tubes under liquid petrolatum. The blood was drawn from twelve to twenty-four hours after a meal. These tubes were left at room temperature for two hours, and the blood was then centrifugalized. Any tubes showing hemolysis or undue turbidity were discarded. The remaining serum was pooled, and a complete spectrophotometric curve was made covering not only the region of absorption of bilirubin but also that of hemoglobin.

As recorded by Sheard, Mann and Bollman,[6] this bilirubin is insoluble in serum without the addition of alkali. We followed a slightly different procedure than they. We dissolved 25 mg. of purified bilirubin in 25 cc. of hundredth-normal sodium hydroxide. This amount was then quickly dissolved in 50 cc. of serum, and any tendency toward alkalinity was neutralized by the addition of 25 cc. of hundredth-normal hydrochloric acid. Owing to the presence of the serum, these stock solutions did not keep indefinitely, but rather became turbid after remaining in the icebox. Dilutions were immediately made from these stock solutions, however, both serum and salt solution being used.

The spectrophotometric curve of bilirubin dissolved in serum differs from that of bilirubin dissolved in chloroform. Possibly this is because in chloroform the bilirubin is present as such, while in the serum it may be present as a salt. At any rate, bilirubin in serum has an absorption band in the visible spectrum with a point of maximum absorption between 460 and 470 millimicrons with a lesser peak between 420 and 430 millimicrons and a depression in the hump in the region of 440 millimicrons. In the ultraviolet end of the spectrum, there is a marked difference in the two curves. The solution of chloroform has its band of maximum absorption in the region of 250 millimicrons, while the solution of serum has its maximum absorption in the region of 310 millimicrons. The point of minimum optical density in the ultraviolet in the solution of chloroform occurs around 340 millimicrons and is rather low. In the dilution of serum it occurs at 370 millimicrons and is relatively high. Chart 2 illustrates the differences in these curves both in the ultraviolet and in the visual up to 510 millimicrons.

We note that this curve does not agree with the observations of Sheard, Mann and Bollman [5] who give an increasing density for bilirubin in alkaline serum up to 430 millimicrons. These same observers with their co-workers find that bile and jaundiced serum have an increasing density up to 430 millimicrons and even to 420 millimicrons, at which point their curves end. Occasionally, we have found a dilution of bile from the gallbladder which apparently has a greater absorption at 440 than at 450 millimicrons. We have uniformly found that normal serum, jaundiced serum and jaundiced ascitic fluid have their maximum absorption at 450 millimicrons.

We have also determined the absorption ratio for bilirubin dissolved in serum at 450 millimicrons. In all figuring it was necessary to take account of the effect of the turbidity and the bilirubin present in the normal serum used as the solvent and diluent. Two separate methods of doing this were employed. The simplest method was to fill one cup of the spectrophotometer with the solution to be examined and to fill the other cup with the diluting serum. As all of the dilutions we studied in the spectrophotometer contained from 96 to 99.2 per cent of this serum, the error due to varying amounts of serums was negligible. The other method we used was to fill one cup of the spectrophotometer with the solution to be examined and the other cup with water. This necessitated making an examination of the serum and subtracting from every reading for the unknown solution a calculated correction for the presence of the serum. The results from these two methods were comparable. Dilutions ranging from 2 to 20 mg. per liter were prepared from three separate stock solutions, and the absorption ratio of $A = 10.88$ was determined for a wave length of 450 millimicrons. On eighteen readings there was an average deviation from the mean of 3.25 per cent and a maximum deviation of 8.5 per cent. We would again stress the importance of making readings only on freshly prepared solutions, since these solutions rapidly become turbid and since turbidity markedly increases the density.

An attempt was now made to assign definite values in milligrams of bilirubin to the various dilutions of dichromate. These dilutions were made as outlined by Murphy [14] with indexes ranging from 1 to 100. Wassermann tubes of a uniform internal bore of 12.5 mm. were filled with these solutions, corked, labeled and sealed with paraffin. Thus sealed they keep indefinitely. For reading our indexes we have constructed a small wooden box 17 by 8.5 by 3.5 cm. Six holes, 16 mm. in diameter, were bored in one of the narrower sides. The Wassermann tubes fit snugly into these holes and sit in depressions on the opposite side of the box, thus firmly fixing the tube. Six parallel longitudinal slots 7.5 mm. in width were cut through the wide side of the box, the center of the slot being directly over the midline of the tube. Small wooden partitions separate the spaces for the tubes into compartments. The whole box is painted black. There is no ground glass or other backing to the box, all readings being made against a northern sky by daylight.

Various dilutions of bilirubin in serum were then made containing from 1 to 100 mg. of bilirubin per liter. The approximate icterus index of each of these dilutions was then determined. A more accurate comparison was then made using a Duboscq colorimeter as advocated by

14. Murphy (footnote 1, second reference).

Bernhardt and Maue.[15] In every case the serum was matched as an unknown against an approximately equivalent dichromate solution. The dichromate was set at 10, and the readings for the serum were determined. From this reading the equivalent value of dichromate in icterus index (hundreds of milligrams of dichromate per liter) was determined. In each case, however, it was necessary to make a correction for the amount of bilirubin present in the normal serum before the bilirubin was dissolved. This correction was merely a subtraction of the icterus index of the normal serum previously determined. Thus, potassium dichromate (icterus index 5 containing 500 mg. per liter) set at 10 is matched by synthetic serum containing 6 mg. of bilirubin per liter set at 8.1.

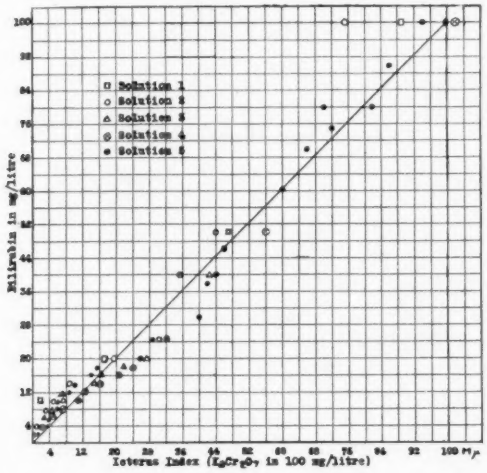

Chart 3.—Graph showing concentration of bilirubin in milligrams per liter plotted as ordinates against dilutions of potassium dichromate plotted as abscissas.

Serum containing 6 mg. bilirubin $= \frac{5 \times 10}{8.1} = 6.17$ icterus index. The normal serum, however, which was used as the solvent and diluent was known to be equivalent to an icterus index of 2.1. Therefore, 6 mg. bilirubin per liter $= 6.17 - 2.1 = 4.07$ icterus index.

Chart 3 is a graph in which the concentrations of bilirubin expressed in milligrams per liter are plotted as ordinates against dilutions of potassium dichromate plotted as abscissas. Five separate solutions of bilirubin in serum were thus matched and fifty-seven points recorded.

15. Bernhardt and Maue, cited by Stetten, DeWitt: The Surgical Value of the Estimation of the Bile Pigmentation (Icterus Index) of the Blood Serum, Ann. Surg. **76**:191 (Aug.) 1922.

It will be noted that a straight line drawn through the maximum number of points is almost an exact diagonal of the graph. In other words, an icterus index of 100 is equivalent to 100 mg. of bilirubin per liter, an icterus index of from 50 to 50 mg. of bilirubin per liter and an icterus index of 16 to 16 mg. per liter. In the weaker dilutions the percentage of error is increasingly higher, and with an icterus index of 6 or below, the error may be marked. It will be seen, however, that the error is greatest in the range of normal serum, and that the comparison becomes increasingly accurate the more jaundiced the serum.

We know that the normal serum has an icterus index of from 3 to 8, usually from 4 to 6. Estimating from our graph, we would assign to these indexes values of from 4 to 10 mg. of bilirubin per liter. This is in accord with what certain other workers have assumed.[16] Why this relationship should obtain between two substances with such different absorption spectra we cannot explain. We do know, however, that this same relationship does not obtain between potassium dichromate and the chloroform solutions of bilirubin. Although dichromate has a greater absorptive power for light than the bilirubin solution with which it matches, it is probable that the absorption is, for the greater part, toward the extreme violet and the ultraviolet end of the spectrum beyond the region of perception by the eye, and that the transmitted light integrates to give the same visual effect.

Turbidity.—Two of the chief obstacles to accurate quantitative determinations of pigment in serum are turbidity and hemolysis. Any method which would eliminate either of these would make for much greater accuracy. Ernst and Förster [17] have apparently used both alcohol and acetone separately to precipitate the proteins and to clear the serum. Magath, Sheard, Mann, Bollman and Baldes [5] have used a mixture of alcohol (4 parts) and acetone (1 part) later changed to 3:1 for the same purpose.

We have tried alcohol and acetone separately, alcohol-acetone mixtures and an alcohol-acetone mixture made alkaline. We have used one or all of these on jaundiced serum, both human and dog, on diluted bile (obtained at autopsy), on bilirubin dissolved in serum and on bilirubin dissolved in chloroform. All of these precipitants reduce the turbidity of serum, but in every case they precipitate a certain amount of pigment. Chart 4 presents graphically our experiences with four of these solutions, namely, jaundiced serum of man and dog, diluted bile and bilirubin dissolved in chloroform.

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16. Förster, J.: Ueber Die normalen Werte Des Bilirubingehaltes im Blutserum, Klin. Wchnschr. **4**:1689 (Aug. 27) 1925.

17. Ernst, Z., and Förster, J.: Ueber die Bestimmung des Blutbilirubins, Klin. Wchnschr. **3**:2386 (Dec. 23) 1924.

In every case the substance examined was diluted with physiologic sodium chloride to a concentration which could be easily read. Identical dilutions were then made with the various mixtures as indicated. All tubes were allowed to stand for two hours and the solution was then centrifugalized; the supernatant fluid was analyzed and the readings were recorded. In most cases the remaining solution was allowed to stand for twenty-two hours more; it was again centrifugalized and analyzed. In every case there was more fading at the end of twenty-four

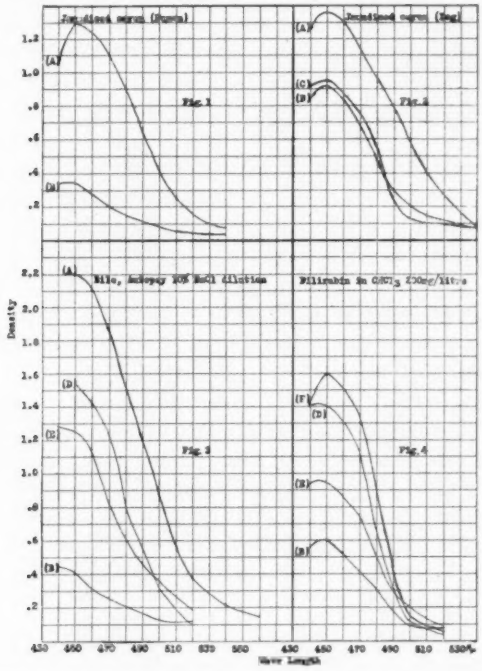

Chart 4.—Curves showing the effect of precipitants on various solutions of bilirubin. *A* indicates the curve when saline was used; *B*, alcohol-acetone; *C*, alkaline alcohol-acetone; *D*, acetone; *E*, absolute alcohol and *F*, chloroform. In figures 1 and 2 the dilution was 1:4; in figure 3, 2:1 and in figure 4, 1:9.

hours than at the end of two hours. That this precipitation is a matter of importance is illustrated in chart 4 (fig. 1), which shows a loss of color in jaundiced serum of over 70 per cent due to the precipitation with the alcohol-acetone mixture. Diluted bile showed a loss of over 80 per cent of the total pigment due to the alcohol-acetone mixture (chart 4, fig. 3). In no case have we found more color lost than that already recorded, in many cases considerably less. In certain instances alcohol

or acetone alone have apparently made little difference (chart 4, fig. 4). In chart 4 (fig. 2) we have also recorded the effect of making the alcohol-acetone mixture distinctly alkaline (1 cc. of tenth-normal alcoholic sodium hydroxide to 100 cc. of alcohol-acetone mixture). It has apparently not altered the fact that the mixture precipitates a large amount of pigment, in this case over 30 per cent. In view of the fact that we have demonstrated the relative insolubility of bilirubin in these various solvents without the addition of alkali, we do not think it at all surprising that this precipitation should occur. In this connection it is interesting to speculate on the accuracy of the van den Bergh reaction, which requires the addition of two volumes of 96 per cent alcohol to one volume of serum to precipitate the proteins before any attempt is made to estimate the "units of bilirubin." [18] Bernheim has also hinted at this inaccuracy.[19] Further comment is made by Perkin.[20]

In their latest communication [5] Sheard, Mann and Bollman say that they have found it necessary to add alkali to the alcohol-acetone mixture when working with the purified bilirubin. They also say that when working with the serums of normal and jaundiced dogs they have added only the alcohol-acetone mixture. They apparently content themselves with the statement that "Tests have demonstrated that the solutions prepared in this manner are slightly alkaline." They do not state whether they have compared the bilirubin content of these dilutions with control dilutions. Using these precipitants, we too have found transmissions of 90 per cent or better (density 0.05 and less) in the region between 500 and 700 millimicrons, but by this method we have precipitated from 30 to 80 per cent of the pigment. This error is many times greater than that due to the turbidity present in carefully collected serum.

Effect of Hemolysis.—We cannot agree with the statement of Brown [21] that slight hemolysis does not vitiate the icterus index test. We have taken jaundiced serum and made various saline dilutions giving an icterus index range of from 1 to 50. Five cubic centimeters of these dilutions was pipetted into our icterus index tubes. These were read and recorded. The blood of a normal dog was taken in liquid oxalate such that it contained 9 Gm. of hemoglobin per hundred cubic centimeters. A 10 per cent hemolyzed solution of this was made in 0.4 per cent ammonia. Then a dropper freeing 25 drops per cubic centimeter was

18. Van den Bergh, A. A., and Snapper, J.: Die Farbstoffe des Blutserums, Deutsches Arch. f. klin. Med. **110**:540 (May) 1913.

19. Bernheim, A. R.: The Significance of Variations of Bilirubinemia, Arch. Path. **1**:747 (May) 1926.

20. Perkin, F. S.: Blood Bilirubin, Estimation and Clinical Significance, Arch. Int. Med. **40**:195 (Aug.) 1927.

21. Brown, A. L.: Rapid Clinical Method for Determination of Icterus Index, Arch. Path. **3**:409 (March) 1927.

used, a drop of blood being placed in each tube and the icterus index read. Additional drops were added one at a time to each tube until the hemolysis could be easily seen. The latter point, being a matter of individual decision, is subject to wide variation, and although at the points we have decided on there are relatively large amounts of hemoglobin, we doubt if the ordinary observer would detect amounts much below these points. Frequently before we could detect any hemolysis grossly we could determine that the solution was definitely off color, but below this point the addition of hemoglobin simply raised the icterus index reading of different dilutions. This is graphically expressed in table 2. It is interesting to note that although the hemolysis is first noticed in the lower ranges, from icterus index 1 to icterus index 5, it affects the readings in this region little if at all. Above icterus index 7,

TABLE 2.—*Icterus Indexes of Various Dilutions of Jaundiced Serum With and Without the Addition of Hemolyzed blood (1 Drop Blood = 0.007 Per Cent)*

Icterus Index of Serum	Serum Plus 0.007% Hemoglobin	Serum Plus 0.014% Hemoglobin	Serum Plus 0.021% Hemoglobin	Serum Plus 0.028% Hemoglobin	Serum Plus 0.035% Hemoglobin	Hemolysis Evident, per Cent of Hemoglobin
1-2	2	2	*	..	..	0.028
2-3	**2-3**	2-3	2-3	*	..	0.035
3-4	3-4	3-4	3-4	*	*	0.042
4-5	4-5	4-5	4-5	*	*	0.049
7-8	8-10	10-12	10-12	*	*	0.063
8-10	12-15	12-15	12-15	15-20	*	0.077
12-15	15-20	20-25	25	35	*	0.084
15	15-**20**	25-35	25-35	35-50	*	0.084
15-20	20-**25**	35	35-50	50-75	75-100	0.084
20-25	**25**-35	35-50	50	75-100	100	0.084
25-35	**35**-50	50-75	75	100	100	0.077
35-50	35-**50**	50-75	75-100	100	100	0.076

* Solution off color.
Bold faced figures denote the nearest index.

however, the readings are increased even with one drop of the 10 per cent solution of hemolyzed blood which is a concentration of hemoglobin of 0.007 per cent (1:14,000).

Kennedy [22] has recently discussed the value of light filters in colorimetry. As two of the absorption bands of hemoglobin occur in an entirely different part of the spectrum, 540 and 575 millimicrons, it was thought that if the solutions could be compared through a light filter which would cut out rays of a greater wave length than 500 millimicrons and yet have a high transmission in the region of 450 millimicrons, at least a part of the effect due to the hemoglobin could be ruled out. The Wratten light filter no. 47 made by the Eastman Kodak Company seemed to fulfil these requirements. It is a blue filter which effectively cuts out

22. Kennedy, R. P.: The Use of Light Filters in Colorimetry with a Method for the Estimation of Hemoglobin, Am. J. Physiol. **78**:56 (Sept.) 1926.

the bands of hemoglobin at 540 and 575 millimicrons. There is another band of hemoglobin the same as bilirubin in the region 450 millimicrons. Obviously, no light filter can be expected to differentiate between this band and the band of bilirubin. Our only objection to filter no. 47 is that even at 450 millimicrons there is an optical density of approximately 0.3. Dr. Mees kindly provided us with a special blue filter 846-2 which is as effective in removing the lines of hemoglobin, and which at the same time has a density of approximately 0.1 in the region of 450 millimicrons. Chart 5 illustrates how light filter 846-2 excludes light rays in the region of two of the bands of hemoglobin, while giving a high transmission in the region of maximum absorption of bilirubin. In reading the solutions it apparently made little difference whether the filter was held in front of the eye with the solutions at arms length or whether the filter was

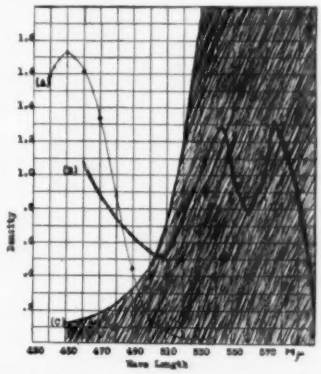

Chart 5.—Graph showing how light filter excludes light rays in the region of two bands of hemoglobin but yet has high transmission in region in which bilirubin has great absorption. *A* indicates bilirubin in chloroform; *B*, oxyhemoglobin and *C*, light filter 846-2.

placed in the box just behind the tubes. In either case, the solutions appear as blue to green, depending on the amount of the yellow pigment present.

In table 3, we show the effectiveness of comparing solutions through these filters. Column 1 shows the icterus index of the saline dilutions of the serum; column 2 the icterus index of the different dilutions with the addition of three drops of the solution of hemolyzed blood; column 3 the indexes read through filter 846-2, and column 4, the indexes read through filter 47.

There appears to be little choice between these two filters except in the upper ranges, where with the dark solutions the greater density of no. 47 is a disadvantage. It will be noted that in every case in which hemolysis had increased the icterus index reading, the filters have

restored it almost to normal and at the same time have not changed the readings which had not been increased by hemolysis. These filters have a definite but limited value. We strongly advise against the attempt to read the icterus index of any serum showing grossly evident hemolysis.

SUMMARY AND CONCLUSIONS

1. The solubilities and spectrophotometric qualities of bilirubin in various solvents have been studied.

2. Various solutions of potassium dichromate are compared with solutions of purified bilirubin dissolved in serum. It was found that an

TABLE 3.—*Icterus Indexes of Various Dilutions of Jaundiced Serum Before and After Addition of 3 Drops of Hemolyzed Blood Read With and Without Filters*

Icterus Index of Serum	Serum Plus 0.021% Hemoglobin	Filter Number 840-2	Filter Number 47
4–5	4–5	4–5	4–5
7–8	10–12	7–8	7–8
8–10	12–15	**10**–12	**10**–12
12–15	25	12–15	12–15
15	**23–35**	15	15
15–20	35–50	20–25	20–25
20–25	50	**25–35**	**25–35**
25–35	75	**35–50**	**35–50**
35–50	75–100	50	50

Bold faced figures denote the nearest index.

icterus index of 100 matches roughly a synthetic jaundiced serum containing 100 mg. of bilirubin per liter and that weaker dilutions are nearly proportional.

3. In an attempt to remove the turbidity of jaundiced serum by the addition of alcohol-acetone mixture, it is frequently found that from 60 to 75 per cent of the bilirubin is precipitated.

4. It is shown that amounts of hemolysis which cannot be detected by the eye greatly increase the icterus index readings unless a light filter is used.

5. With specific blue color filters it is possible to determine fairly accurately the icterus index of serums containing a relatively large amount of hemolysis.

URINARY PROTEINS NOT ORIGINATING IN BLOOD

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The origin of the albumin in the urine is intimately concerned with the fundamental metabolic disturbances of nephritis. Immunologic studies of these proteins have thus far proved almost totally unproductive on account of the fact that serum proteins are usually present in albuminurias, and the separation of this blood serum fraction has thus far proved impossible.

Aside from the Bence-Jones protein which is probably not concerned in the problem of nephritis, pure urinary proteins have only been prepared in a few isolated cases in which crystallization took place. Such cases have been reported by Paten,[1] Bayne-Jones [2] and Hektoen, Kretschmer and Welker,[3] and a large series of cases by Welker, Thomas and Hektoen.[4] Excretion of protein-containing urine free from blood proteins has never been reported. In the cases quoted, the workers were unable to extract any proteins from tissues which reacted with antiserums prepared for the urinary proteins.

The surest method of identification of the proteins is the immunologic technic already made use of by Hektoen and Welker.[5] Rabbits are sensitized by the injection of blood proteins, and their serums will show positive precipitin reactions in dilution of 1 to 500,000 to this protein. Except as stated before, it has hitherto been impossible to prepare pure urinary proteins free from serum proteins, and therefore these immunologic methods have failed to identify their sources.

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1. Bramwell, B., and Paten, D. N.: On a Crystalline Globulin Occurring in Human Urine, Reports of Lab. Roy. Coll. Phys., Edinburgh, vol. 4, pp. 4, 7 and 1092.

2. Bayne-Jones, S.; Wilson, D. W., and Everett, H. S.: Precipitin Reactions of Crystalline Globulin from Human Urine, Bull. Johns Hopkins Hosp. **34**:77 (July) 1923.

3. Hektoen, Ludvig; Kretschmer, H. L., and Welker, W. H.: A Peculiar Crystalline Protein in Human Urine, J. A. M. A. **83**:1154 (Oct. 11) 1924.

4. Welker, W. H.; Thomas, W. H., and Hektoen, Ludvig: Urinary Proteins—Crystalline Proteins of Nephritis, J. A. M. A. **86**:1333 and 1334 (May 1) 1926.

5. Hektoen, Ludvig, and Welker, W. H.: The Precipitin Reaction of Fibrinogen, J. A. M. A. **85**:434 and 435 (Aug. 8) 1925.

In a previous report, one of us (Andrews)[6] showed that the characteristic uremic syndrome can be produced by the injection of hypertonic salt solutions into animals made acid by the absorption of autogenous edema fluid. The clinical, chemical and histologic picture of uremia is accurately reproduced by this experimental method. In these experiments profound pathologic changes in the liver cells were noted, a chloride concentration higher in the liver than in the other organs, and evidence of the presence of an extreme distortion of the calcium-potassium ration. It was suggested that as a result of a great increase

TABLE 1.—*Proteins in Urine of Dog in Uremia (Whole Urines)*

Experiment	Protein		Precipitin Reaction—Serum 1,500,000							
	Biuret or Heller	Benzidine	Undiluted	1:10	1-50	1:100	1:1,000	1:50,000	1:100,000	1:500,000
1-A	+	0	+	0	0	0	0	0	0	0
B	+	0	+	+	0	0	0	0	0	0
C	+	0	+	+	0	0	0	0	0	0
D	+	0	+	+?	0	0	0	0	0	0
E	+	0	+	+	0	0	0	0	0	0
2-A	+	0	+	+	?	0	0	0	0	0
B	+	0	+	+	0	0	0	0	0	0
C	+	0	+	+	0	0	0	0	0	0
D	+	0	+	+	0	0	0	0	0	0
E	+	0	+	+	0	0	0	0	0	0
F	+	0	+	0	0	0	0	0	0	0
3-A	+	0	+	0	0	0	0	0	0	0
B	+	0	+	0	0	0	0	0	0	0
C	+	0	+	+	0	0	0	0	0	0
D	+	0	+	+	+	0	0	0	0	0
E	+	0	+	+	+	0	0	0	0	0
F	+	0	+	+	+	0	0	0	0	0
G	+	0	+	+	+	0	0	0	0	0
H	+	0	+	+	0	0	0	0	0	0
I	+	0	+	+	+	0	0	0	0	0
4-A	+	0	+	0	0	0	0	0	0	0
B	+	0	+	0	0	0	0	0	0	0
C	+	0	0	0	0	0	0	0	0	0
D	+	0	0	0	0	0	0	0	0	0
E	+	0	0	0	0	0	0	0	0	0
F	+	0	+	0	0	0	0	0	0	0
G	+	0	+	0	0	0	0	0	0	0
H	+	0	+	0	0	0	0	0	0	0

in the permeability of the liver cells soluble proteins were allowed to leak into the blood stream, and that they were excreted in the urine. It has also been assumed that passage of foreign proteins through the kidney epithelium will eventually bring about a leakage of serum proteins as well.

With these points in view, studies were undertaken of the proteins in the urines of dogs in artificial uremia, brought about as described by Andrews.[6] Two rabbits were sensitized to dog blood protein by the method previously described so that they reacted positively in dilutions of 1 to 500,000.

6. Andrews, Edmund: Artificial Uremia, Arch. Int. Med., to be published.

The urinary proteins were obtained by catheter from female dogs. Specimens were collected at short intervals and kept separate. Heller's test on each sample was later confirmed by Biuret tests. Numerous samples were spoiled by the presence of traumatic blood as evidenced by positive benzidine tests. Table 1 is a summary of those results in specimens which were blood-free and still gave strong protein reactions. The negative precipitin reactions, even undiluted in some cases, showed

TABLE 2.—*Proteins in Urine of Dog in Uremia (1 Per Cent Solution of Purified Proteins)*

Experiment	Protein: Biuret or Heller	Benzidine	Precipitin Reaction—Serum 1,500,000: Undiluted	1:10	1:50	1:100	1:1,000	1:50,000	1:100,000	1:500,000
A	+	0	0	0	0	0	0	0	0	0
B	+	0	0	0	0	0	0	0	0	0
C	+	0	?+	?	0	0	0	0	0	0
D	+	0	0	0	0	0	0	0	0	0
E	+	0	+	+	0	0	0	0	0	0

TABLE 3.—*Proteins in Urines of Dogs Under Ether Anesthesia*

Dog	Time	Heller or Biuret	Benzidine	Precipitin Reaction—Serum 1,500,000: Undiluted	1:10.	1:50	1:100	1:1,000	1:50,000	1:100,000	1:500,000
B	1:30	+	0	+	+	0	0	0	0	0	0
	2:40	Trace	0	+	0	0	0	0	0	0	0
	3:00	Trace	0	+	0	0	0	0	0	0	0
	3:20	Trace	0	+	0	0	0	0	0	0	0
A	2:10	++	+	+	+	+	+	+	0	0	0
	3:00	++	+	+	+	+	+	+	0	0	0
C	2:00	0	0	0	0	0	0	0	0	0	0
	2:30	0	0	0	0	0	0	0	0	0	0
	3:00	+	0	+	+	0	0	0	0	0	0
	3:30	++	0	+	+	0	0	0	0	0	0
D	2:00	+	0	+	+	0	0	0	0	0	0
	2:30	+	0	+	+	0	0	0	0	0	0
	3:00	+	0	+	+	0	0	0	0	0	0
E	2:00	+	0	+	0	0	0	0	0	0	0
	2:30	+	0	+	0	0	0	0	0	0	0
	3:00	+	0	+	0	0	0	0	0	0	0
F	3:00	+	0	0	3	0	0	0	0	0	0
	3:15	+	0	0	0	0	0	0	0	0	0
	3:45	+	0	0	0	0	0	0	0	0	0
G	3:15	++	0	+	+	0	0	0	0	0	0
	4:45	++	0	+	+	0	0	0	0	0	0
H	10:15	+	0	0	0	0	0	0	0	0	0
	10:30	+	0	+	+	0	0	0	0	0	0

clearly that these were not blood proteins leaking through. This applies only to the early samples. Later ones were invariably contaminated with serum proteins. Precipitin tests were negative, not only with the whole urine but also with 1 per cent solutions of purified proteins from those urines, as shown in table 2.

Next a series of similar tests made using the first samples of protein-containing urine brought about by etherization, as ether is a liver poison (table 3), shows that in this case also the earlier specimens are not blood proteins, but later blood proteins appear.

SUMMARY

These experiments demonstrate clearly that in the early stages of albuminuria a protein foreign to the blood proteins is excreted, and that after a short time the renal epithelium is rendered permeable to normal serum proteins.

NOTE: These studies are being continued by the preparation of rabbit serums sensitized to the blood-free urinary proteins and also to normal and uremic liver proteins, and other organ proteins, in order to ascertain, if possible, the source of the urinary proteins.

Book Reviews

SEGREGATION AND AUTOGAMY IN BACTERIA: A CONTRIBUTION TO CELLULAR BIOLOGY. By F. H. STEWART, M.D., Major, Indian Medical Service (Ret.). Paper Boards. Price, 7 shillings, 6 pence. Pp. 104. London: Adlard & Son, Ltd.

Among the most fascinating problems in the study of infectious diseases are variations in virulence. To the physician, the microbiologist and the epidemiologist each new contribution to the study of microbic variation holds out the encouragement that perhaps the veils behind which the mysteries hide will be parted a little wider. But little more than disappointment comes from an examination of this newest monograph. Beginning essentially where Gurney Dixon left off nearly ten years ago, the author presents an analysis of variations in certain cultures of bacteria — particularly of variations associated with fermentation characteristics — which adds little of probable importance or permanent worth.

By a series of ingenious arguments, the author demonstrates that certain variations in culture may be described by assuming that the variant characteristics are determined by multiple allelomorphs. He then proceeds to the experimental "demonstration" of segregation and perhaps autogamic conjugation, and concludes by deducing a probable "life history" of a bacterium.

It is rather a pity that a serious investigator of bacterial variation proceeds with complete indifference to the overwhelming evidences that plate colonies (no matter how many times repeated) are not necessarily pure cultures, and to the value of beginning with single cell strains. Furthermore, although certain of the types of variations Dr. Stewart describes are of unquestioned occurrence, there is almost complete neglect of all the recent experimental and speculative literature on "S — R" variations in bacteria with their associated variations in fermentative, morphologic, virulence, antigenic and other characteristics. Wider familiarity with bacteriologic literature would probably force the author to dispense with his primary division of continuous and discontinuous, permanent and non-permanent variations (cf. Philip Hadley, J. Infect. Dis. **40**:1, 1927).

The present monograph does not significantly modify the view generally held that the genetic interpretation of variations in the bacteria must await a more complete understanding of the determining factors than is now available.

DISEASES OF THE SKIN. By HENRY H. HAZEN. Price, $10. Pp. 572, with 248 illustrations, including 2 color plates. St. Louis: C. V. Mosby Company, 1927.

The medical student and practitioner will find in this conveniently sized work, accurate, concise, useful information regarding the more common dermatologic subjects. Dermatoses of the negro are given special consideration, making the text valuable to those who have occasion to treat colored patients.

Preliminary consideration is given to anatomy and physiology, etiology, symptomatology, pathology, diagnosis and general aspects of therapy. Among the more timely chapters are those on general hygiene of the skin, emotional nervous disorders, carcinoma, and the public health aspects of syphilis. Actinotherapy and diathermy are given attention. Microscopic observations of the more common dermatoses are briefly presented throughout.

The author avoids discussion of the relative merits of the terms "eczema" and "dermatitis," and employs only the latter. He forsakes the dermatologists' heritage of "objective examination" by recommending history taking before examination.

Owing to the size of the book, little space is devoted to antisyphilitic therapy. This phase has become so important that it is questionable whether it should be included in dermatologic texts. For instance, the author's "average course" of treatment for early syphilis contains, in the light of recent studies, too long (three months) mercurialization between courses of arsphenamine. The advantages of arsphenamine over neoarsphenamine are emphasized.

The clinical illustrations are numerous and are of convenient size; part of them are of colored patients; most of them are good and many are excellent. The drawings and photomicrographs, few in number, are of little value.

The book is not padded by a bibliography, although pertinent publications are mentioned in the text.

THE COMPARATIVE PHYSIOLOGY OF INTERNAL SECRETION. By LANCELOT T. HOGBEN, M.A. (CANTAB.), D.Sc. (LOND.), Professor of Zoology in the University of Capetown. Price, $3. Pp. 148, with 37 illustrations and index. New York: The Macmillan Company, 1927.

This is a well written and critical statement of the comparative physiology of the endocrine glands. The arrangement of the material is from the point of view of systems in the body influenced by different hormones rather than the usual descriptions of syndromes following hypoactivity or hyperactivity of the glands. The sound critical attitude of the author may be indicated by the following quotations from the initial chapter headed "Chemical Co-Ordination": "In pursuing inquiry into the endocrine or supposedly endocrine function of so-called ductless glands the physiologist has no more justification for attributing a teleological significance to every chemical entity in the organism than neo-Darwinian naturalists had for ascribing utility to every member of the body." That is to say, the author clearly recognizes that the finding of substances by various chemical treatments of organs, substances that have various physiologic actions when introduced directly into the blood or under the skin, does not mean that these substances necessarily are produced by these organs and have these actions under normal conditions of life.

There are chapters on epinephrine and neuromuscular coordination, on the internal secretions as related to chromatic function, the endocrine factors in secretory processes, the endocrine factors in vasomotor regulation, the endocrine factors in metabolism and the endocrine factors in growth.

The author is especially conversant with the experimental literature on the endocrine glands in lower animals. Equal familiarity with the experimental and clinical literature on endocrines in the higher animals is, of course, a prerequisite in order to treat endocrinology in a comparative way. In most cases the author measures up to this requirement. The weakest chapter in an otherwise excellent monograph is that of the influence of the endocrines in secretory processes. Edkins' experiment on gastrin (1906) is quoted and discussed, while more conclusive experiments, especially those of Ivy, Lim, Luckhardt, Koch and Keeton are not referred to, although these experiments alter Edkins' original interpretation, if not the facts themselves.

The monograph is to be regarded as a valuable contribution to the subject of endocrinology and may be pursued with profit by all physicians interested in this field.

NERVE TRACTS OF THE BRAIN AND CORD: ANATOMY, PHYSIOLOGY, APPLIED NEUROLOGY. By WILLIAM KEILLER, F.R.C.S., Professor of Anatomy and Applied Anatomy, University of Texas. Price, $8. Pp. 456. New York: The MacMillan Company, 1927.

According to the intentions of the author this book will enable students to approach nervous diseases, thinking in terms of anatomy, physiology and pathology. The book consists of three parts: part 1 (pp. 3 to 113), a laboratory

manual for the study of the central nervous system under normal and pathologic conditions based on Weigert and Marchi preparations; part 2 (pp. 117 to 178), a summary of the anatomy and physiology of the nerve tracts, mainly based on newer methods of investigating the observations found at autopsy in chemical cases; and part 3 (pp. 179 to 329), the more important features of the better known nervous diseases, correlating their symptomatology with anatomic, physiologic and pathologic data.

Pages 333 to 436 contain a great many illustrations, and there is a large diagram showing the chief fiber tracts of the brain, brain stem and spinal cord.

As a matter of fact, the book contains a vast amount of diversified knowledge. The author shows a surprisingly great familiarity with the anatomy of the central nervous system. It is therefore to be regretted that the facts are presented in such a way as to confuse even the more experienced reader. The three parts appear to the student as three books which deal to some extent with the same matter. Thus it comes about that there are many subjects discussed two and even three times, often in a similar style. Since most of the discussions are short and not detailed, one regrets that this unnecessary repetition occurs at the expense of the completeness of the representation. For instance, the physiology of the cerebellum is described in the first part on pages 21 and 22; in the second part on pages 169 and 170; and in the third part on page 261. Even if the points of view from which parts of the nervous system are considered may differ somewhat in the three parts of the book, the whole arrangement is rather confusing. Furthermore, the use of sketches and rough drawings does not seem to be the best method of illustration for a book of this type.

AFFECTIONS OF THE STOMACH. By BURRILL B. CROHN, M.D., Associate Attending Physician to the Mt. Sinai Hospital, New York City. Cloth. Price, $10 net. Pp. 902, with 361 illustrations, some in colors. Philadelphia: W. B. Saunders Company, 1927.

The first 165 pages are devoted to the normal anatomy and physiology of the stomach, including a detailed description of the standard test meals and a discussion of their interpretation. The subject of gastric radiography is well handled and adequately for the use of the average general practitioner or internist, in 80 richly illustrated pages. The essential points in the gastric history as well as those of the physical examination of the patient are carefully pointed out.

The author devotes 215 pages to the classification, description and discussion of nonorganic diseases of the stomach including dyspepsia, achylia gastrica, gastroptosis, gastric manifestations of reflex origin, gastric neuroses and similar conditions. While there is much of good in these chapters on important and difficult subjects about which comparatively little has been definitely proved, many readers will probably be disappointed in them. The fault lies perhaps not so much with the author, as it does with the entirely inadequate knowledge of this whole group of subjects.

The 332 pages devoted to definite organic diseases of the stomach are by far the best in the book. Ulcer of the stomach and duodenum is carefully considered and thoroughly presented. The various types of medical and surgical treatment are described, and an attempt is made to evaluate them. The standpoint of the Mount Sinai group is given. The benign and malignant tumors of the stomach receive adequate attention, and the question of differentiating clinically between gastric ulcer and gastric carcinoma is dealt with fully. The chapter on organic syphilis of the stomach will interest many. The book ends with a discussion of herniation of the stomach through the diaphragm.

NUTRITION AND DIET IN HEALTH AND DISEASE. By JAMES S. MCLESTER, M.D., Professor of Medicine, Graduate School of Medicine, University of Alabama, Birmingham, Ala. Cloth. Price, $8 net. Pp. 783. Philadelphia: W. B. Saunders Company, 1927.

This comprehensive treatise leaves nothing more to be desired in a book of its type. Normal diets and normal nutritional requirements for adults and for children of the preschool age are thoroughly considered. The feeding of infants is discussed in a special chapter by Dr. McKim Marriott of Washington University, St. Louis, and the feeding of surgical patients in a chapter written by Dr. Barney Brooks of Vanderbilt University, Nashville.

In the sections on diet in disease, the author does much more than give the various empiric diets that have been used; he discusses the nutritional, metabolic and digestive disturbances involved and then proceeds to outline their dietary correction. Menus, recipes, tables and charts are numerous throughout the book, serving to enhance its value as a reference text for students and practitioners.